University of South Alabama
JagWorks@USA

Theses and Dissertations | Graduate School

12-2021

Variability in the Condition of Fundulus Grandis Across Alabama's Coastal Waters: A Potential Indicator of Ecosystem Health

Nicholas A. LaBon
University of South Alabama, nal1821@jagmail.southalabama.edu

Follow this and additional works at: https://jagworks.southalabama.edu/theses_diss

 Part of the Pharmacology, Toxicology and Environmental Health Commons

Recommended Citation

LaBon, Nicholas A., "Variability in the Condition of Fundulus Grandis Across Alabama's Coastal Waters: A Potential Indicator of Ecosystem Health" (2021). *Theses and Dissertations*. 12.
https://jagworks.southalabama.edu/theses_diss/12

This Thesis is brought to you for free and open access by the Graduate School at JagWorks@USA. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of JagWorks@USA. For more information, please contact mduffy@southalabama.edu.

VARIABILITY IN THE CONDITION OF *FUNDULUS GRANDIS* ACROSS ALABAMA'S COASTAL WATERS: A POTENTIAL INDICATOR OF ECOSYSTEM HEALTH

A Thesis

Submitted to the Graduate Faculty of the
University of South Alabama
in partial fulfillment of the
requirements for the degree of

Master of Science

in

Environmental Toxicology

by
Nicholas A. LaBon
B. S., Augusta University, 2016
December 2021

ACKNOWLEDGMENTS

There are many people I would like to thank who helped me through this project. First, I would like to thank my committee: Dr. Coym, Dr. Robertson, and especially my committee chair Dr. Baker. Thank you for sticking with me and guiding me as I developed as a scientist. I would like to thank Mai Fung for assisting me with the spring killifish collections and Michael Bradley for providing the code for generating CARTs in R. I would also like to thank Dr. Duranty for teaching me how to use a bomb calorimeter. Next, I would like to thank Alex Rodriguez and Sarah Ramsden for help with peer review of this thesis. Lastly, I would also like to thank my wife, Jessica, for keeping me on track, reminding me of deadlines, and accompanying me on some of the killifish collections. Thanks again to everyone who gave me support along the way.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

LIST OF ABBREVIATIONS

AIC	Akaike Information Criterion
AIR	Airport Marsh
ANCOVA	Analysis of Covariance
ARCOS	Alabama Real-Time Coastal Observation System
ARL	Arlington Park
BACI	Before-After Control-Impact
BSRW	Bon Secour Weather Station
CART	Classification and Regression Tree
CFM	Car Ferry Marsh
CED	Cedar Point
CEDW	Cedar Point Weather Station
CPUE	Catch Per Unit Effort
DISLW	Dauphin Island Sea Lab Weather Station
DO	Dissolved Oxygen
FTM	Fort Morgan
FOW	Fowl River
GSI	Gonadosomatic Index
HSI	Hepatosomatic Index

IACUC	Institutional Animal Care and Use Committee
IPC	Industrial Port Canal
LSI	Lipo-somatic Index
MBLW	Mobile Bay Lighthouse Weather Station
MEA	Meaher Park
MEAW	Meaher Park Weather Station
OYB	Oyster Bay
PIN	Pinto Island
PCL	Point Clear
SBR	Shell Belt Road
TEBI	Total Energy Bodies Index
WBN	Weeks Bay North
WBNW	Weeks Bay North Weather Station
WBS	Weeks Bay South
WBSW	Weeks Bay South Weather Station
WEB	West End Beach
WOL	Wolf Bay
WWP	Wade Ward Park

ABSTRACT

LaBon, Nicholas A., M. S. in Environmental Toxicology, University of South Alabama, December 2021. Variability in the Condition of *Fundulus Grandis* Across Alabama's Coastal Waters: A Potential Indicator of Ecosystem Health. Chair of Committee: Ronald Baker, Ph.D.

Monitoring indicator species can be a useful way of assessing the effects of multiple interacting stressors on ecosystem health. As a widespread, ecologically important species, with individuals showing high site fidelity, the Gulf killifish, *Fundulus grandis*, has potential to be a good indicator species of environmental health in coastal regions in the Gulf of Mexico. This study investigated variability in *F. grandis* body condition, including length to weight ratio, hepatosomatic index, gonadosomatic index, liposomatic index, total energy bodies index (developed in this study), and caloric content, in relation to natural environmental gradients, catchment land use, and local seascape composition, within coastal Alabama waters. *F. grandis* were collected from 14 sites across environmental and urbanization gradients across coastal Alabama. *F. grandis* tended to be lighter than predicted for their length at low salinity sites in the upper Mobile Bay, and had a lower mass of energy bodies in sites with more urbanization within the local catchment. While caloric content seemed promising as a condition metric, complications arising from methodology resulted in inconclusive data. Overall, *F. grandis* is a viable indicator species for environmental health within the coastal regions of the Gulf of Mexico.

CHAPTER I

GENERAL INTRODUCTION

1.1 Mobile Bay Estuary

The health of an estuary system can greatly impact the livelihood of a coastal community (Environmental Health Center, 1998). Not only are a large proportion of economically valuable fish considered estuary dependent (Chambers 1992), but healthy estuaries also provide a range of other ecosystem services. These services include providing complex habitats that support many species (McLusky and Elliott 2004), providing some natural filtration of runoff (Zu Ermgassen et al. 2013), and recreational services like boating, fishing, and swimming.

Estuary health can be influenced by terrestrial runoff that delivers contaminants such as excess nutrients (Arismendez et al. 2009; Yang 2012), heavy metals (Sanger et al. 1999, Holland et al. 2004), pesticides (Sanger et al. 2004), and other pollutants (Van Dolah et al. 2008) to the estuary system. Once the water slows when it enters the estuary, many contaminants can accumulate in sediments or biota, combining to potentially degrade the health of the estuary. Detecting the presence of and monitoring how these contaminants affect the health of an estuary is necessary to ensure that the estuary remains healthy and stable.

Mobile Bay is a large estuary at the terminus of 5 major rivers: Mobile, Spanish, Tensaw, Apalachee, and Blakeley Rivers (Fig. 1.1). Smaller rivers also flow into the Bay – Dog, Fowl, and Deer rivers from the west and the Fish River from the east (Fig. 1.2). Mobile Bay has the 4th largest watershed in the contiguous United States in terms of flow and 6th in terms of area (Alexander et al. 2001). It is also one of the shallowest bays for its size with an average depth of 3 m excluding the shipping channel (Coogan and Dzwonkowski 2018).

There is a north to south salinity gradient along Mobile Bay. The bay starts as freshwater discharge from the major rivers at the northernmost point, transitioning to higher but variable salinity when it reaches the Gulf of Mexico (Fig. 1.2). The gradient between the endpoints is caused by wind mixing the surface waters to create a salt wedge gradient (Park et al. 2007, Coogan and Dzwonkowski 2018). There is a turbidity gradient in Alabama coastal waters from east to west (Coogan and Dzwonkowski 2018). The turbid waters in Mississippi Sound and Mobile Bay start to become clear further east towards Perdido River.

The large catchment, coupled with growing urban and industrial development in Mobile and Baldwin counties, means that a variety of pollutants are deposited into the Bay. Pollutants come from numerous point and non-point sources – such as agricultural, industrial effluent, wastewater, and septic tanks – making determining the source of a specific contaminant a difficult task (Baya et al. 1998).

1.2 Water Quality Assessment Considerations

Water quality is assessed in many ways depending on the goal. For instance, water treatment facilities directly test water properties such as hardness, pH, and the presence of contaminants to determine if water is potable by ensuring that any contaminants detected in the water are below levels determined to be safe by the U.S. Environment Protection Agency (EPA).

Safe contaminant levels are established by conducting toxicological studies which determine if specific contaminants can indicate risks to human or other species health. However, without a detailed understanding of the ecological impacts of various contaminants, direct and precise measures of particular contaminants do not provide a clear understanding of ecosystem health or risks (Rice 2003).

Toxicological studies can show the specific effects of a toxin or toxins on a test subject by determining the dose that results in the lowest observed adverse effect level (LOAEL) or no observed adverse effect level (NOAEL) for the test subject (Dorato and Engelhardt 2005). These levels are then used to create regulations and guidelines regarding acceptable levels of certain toxins or contaminants in food, water, or air (Dorato and Engelhardt 2005). While toxicological studies provide useful information on how toxins affect a specific species or group of species, they do not examine how those toxins effect the health of the ecosystem, pathways of exposure, or interactions with other stressors.

One way to assess ecosystem health is by studying a biotic indicator. A biotic indicator shows a measurable response to external environmental condition (Lindenmayer and Likens 2011), and thus can be used to successfully assess the health of

an ecosystem. Biotic indicators can be individual indicator species (Whitehead et al. 2012, Dubansky et al. 2013), or community metrics (Sheaves et al. 2012, Ellis and Bell 2013).

Community metrics such as species composition, relative abundance, and sensitivity to stressors within the community can all provide indications of environmental health (Rice 2003). Environments that contain many different species are usually considered to be in better condition than environments that are dominated by only a few species (Rice 2003). Besides number of species present, whether the species are sensitive to environmental conditions is also important (Qiu and Qian 1998, Dean and Richardson 1999, Gupta and Singh 2011). These sensitive species can include those which are sensitive to low dissolved oxygen (DO) (Dean and Richardson 1999), salinity changes (Qiu and Qian 1998), or pollutants (Gupta and Singh 2011). Observing high numbers of sensitive species can indicate a healthy ecosystem, while the absence of sensitive species can be indicative of stressors or conditions unfavorable for those species.

A community level approach requires a significant investment to obtain data robust enough to detect community responses. Using community metrics to indicate ecosystem health works best when using the before-after control-impact (BACI) sampling design. This approach requires that an impact site and control site be sampled before and after the impact occurs at the impact site. This method accounts for any natural changes that would have occurred at the impact site not caused by the potential impact event (Underwood 1991, Smith 2002). However, in many cases it is not possible to sample the impact site before the impact event. In these cases, a large number of control sites are needed to reflect the conditions of a nonimpacted system due to the high

natural variability in biotic systems (Sheaves et al. 2012). Fish communities are also patchy and require a large sampling effort to accurately represent which species are present and in what relative abundance (Ellis and Bell 2013). So, while community monitoring can provide useful information about ecosystem health, it requires a very large, and often prohibitive amount of sampling, which can make individual indicator species a more attractive option for measuring ecosystem health.

1.3 Using an Indicator Species

An indicator species is a species whose condition, relative abundance, or distribution characterizes the community in which they reside (Lindenmayer and Likens 2011). A good indicator species is one that shows high site-fidelity, shows a measurable response to environmental condition, and is ecologically important (Lindenmayer and Likens 2011).

Showing high site fidelity indicates that the individuals would likely only be exposed to the local environmental conditions at the site they are captured. Without this, any apparent changes in body condition might not be due to environmental conditions at the study site. Wolfe and Lowe (2015) performed a study on the habitat use and site fidelity of the white croaker, *Genyonemus lineatus*, in the Palos Verdes Superfund Site, Los Angeles, California. They determined that while *G. lineatus* has been used as an indicator species for the superfund site, it does not have a high site fidelity and instead is nomadic and doesn't establish a home range. This finding then throws into question the

use of *G. lineatus* as an indicator species since its body condition could be influenced by many different environments and not just the superfund site.

A useful indicator species must show a measurable response to varying environmental conditions, such as physical body condition (Wedge et al. 2015), behavioral change (Fournet et al. 2019), or mortality rates (Bernard et al. 2010). Fournet et al. (2019) demonstrated that the frequency of Gulf toadfish, *Opsanus beta*, calls responds to salinity levels in the Florida Everglades. Males have high nest fidelity and their call frequency is inversely related to salinity. This demonstrates an example of a behavioral response to changing environmental conditions that might be used as an indicator of environmental health.

Lastly, the response of an ecologically important species, such as an ecosystem engineer or key prey or predator species (Lindenmayer and Likens 2011), would characterize their ecosystem better than a species that has little impact on its surrounding community. The term Keystone species, originally coined by Paine (1969), describes a species that has a disproportionately large effect on its surrounding community or environment. If a keystone species suffers due to poor environmental conditions, it is likely that the surrounding community will also be impacted either directly by the same environmental conditions or indirectly due to the strong connections to the keystone species (Mills et al. 1993).

One way to assess the combined effects of pollutants and other pressures on ecosystem health is to monitor the condition of locally-resident indicator species. The Gulf Killifish, *Fundulus grandis*, have been suggested as a useful indicator due to their broad geographic distribution but limited individual movements within estuarine areas

(Nelson et al. 2014, Jensen et al. 2019). *F. grandis* are one of the most abundant nekton species in northern Gulf of Mexico salt marshes (Rozas & Reed 1993, Nelson et al. 2014). They complete their lifecycles within estuaries, with the post-larval individuals primarily occupying flooded marsh habitats for feeding, reproduction, and refuge (Nelson et al. 2014). *Fundulus grandis* are omnivorous (Rozas & LaSalle 1990) but primarily carnivores (Baker et al. 2013) across the size ranges sampled in this study. Very large killifish may be more piscivorous (Harrington and Harrington 1961, Odum and Heald 1972), but few studies have found fish prey to be important for this species. Overall, the diets for fish of the size sampled in this study are dominated by polychaetes, mollusks, amphipods, tanaids, crabs, isopods, grass shrimp, and insects (Rozas & LaSalle 1990).

Fundulus grandis are important in trophic relay. Trophic relay, in this example, is the transfer marsh production to higher trophic levels and other ecosystems that occurs when *F. grandis* get eaten by predators in the open water adjacent to the marsh (Rozas and Reed 1993). This transfer of energy makes *F. grandis* an important link between two ecosystems, making them an ecologically important species in salt marshes and their surrounding open waters.

Fundulus grandis also show high site fidelity. Nelson et al. (2014) conducted a mark and recapture experiment on *F. grandis* to determine the similarity of its home range to its close relative, *F. heteroclitus*. The experiment suggested *F. grandis* exhibit high site fidelity with very few recorded movements greater than 100m. These findings are supported by Jensen et al. (2019) whose batch tagging experiment determined that the short life span and high site fidelity of Gulf Killifish suggests that observed responses to disturbance reflect local conditions (<100m). Their limited movement supports the use of

F. grandis as an indicator species since individuals will have been resident in the immediate environment from which they were collected, meaning the individuals sampled will represent the environmental conditions from the sampling site.

Fundulus grandis and its relatives, like *F. hetetroclitus*, have been used as indicator species in a variety of studies. Burnett et al. (2007) describes *F. heteroclitus* of the Atlantic Coast of North America, and *F. grandis* of the Gulf of Mexico, as premier field and laboratory models for understanding how teleost fishes interact with their environment due to their ability to adapt to a wide range of environments.

Fundulus grandis has only recently become an indicator species in the Gulf of Mexico. It has most commonly been used as an indicator species for salt marshes along the Gulf of Mexico to assess the impact of the 2010 Deepwater Horizon oil spill (Whitehead et al. 2012, Dubansky et al. 2013). Both studies indicated a physiological and reproductive impairment, suggesting population level impacts. These studies show that *F. grandis* might be a viable indicator species for the oil spill, but more studies using *F. grandis* as an indicator species are needed to determine its usefulness as a more general indicator of environmental health.

Fundulus grandis also shares important ecological similarities with its close relative *F. herteroclitus;* it has similar life history and habitat requirements (Kneib and Stiven 1978, Rozas and Reed 1993), and shows high site fidelity (Nelson et al. 2014). These features and the increasing recent use of *F. grandis* in ecotoxicological studies suggest it may be more broadly useful as an indicator species for studies of environmental health and impacts. However, more general tests of its utility as an indicator species are lacking. This study, therefore, will test the utility of *F. grandis* as an

indicator of environmental variability and ecosystem condition. Quantifying variability in killifish condition around Mobile Bay and adjacent Alabama coastal waters can help to identify variations in ecosystem health, identify areas that may be currently degraded, and serve as a baseline to monitor future changes in ecosystem health and function.

1.4 Project Objectives

The overall goal of this thesis is to assess the potential for *F. grandis* to serve as an indicator species of ecosystem health. To achieve this goal, the thesis answers the following questions:

- Does *F. grandis* body condition vary across Alabama's coastal waters? Can this variation be explained by the extent of development in the catchment, local habitat characteristics, and natural gradients of hydrographic properties

By quantifying variability in *F. grandis* body condition across gradients of natural environmental variability and ecosystem health, analyses of spatial variability in condition will seek to identify the key drivers of this variability, such as natural environmental gradients and hot spots of pollution.

1.5 Thesis outline

Chapter II describes and justifies the site selection process, details killifish collection methods, and describes the derivation of physical parameters, local habitat

characteristics, and catchment land use metrics for each study site. It also discusses some challenges in the sampling process. The general methods described in Ch. II provide a foundation from which both chapters III and IV build on.

Chapter III examines variation in morphometric condition indices of killifish among sites. The analyses in this chapter were broken down into two parts because some collection sites were too far from a weather station for reliable hydrographic data (Ch. II). The first set of analysis uses all the sites to look at relationships between killifish condition, and local habitat and catchment land use metrics. The second set of analyses used similar models that included data only from the subset of sites that have hydrographic data available so that hydrographic variables (DO, water temperature, salinity) could be included as explanatory variables.

Because caloric content data were deemed unreliable due to methodological challenges, the use of caloric content as an alternate metric of killifish condition is presented separately in Chapter IV. While caloric content was expected to be a more sensitive measure of killifish condition that accounts for energy that isn't accounted for in other metrics, there were multiple challenges in the process that made the resulting data unreliable. These challenges are described, and remedies for future attempts for using caloric content as a condition metric are suggested.

Chapter V provides a general discussion for the thesis, concluding that killifish condition does show predictable variation across Alabama's coastal waters, and that this variation can be partially explained by both natural gradients (salinity), impacts in the catchment, and local environmental conditions. These measured responses to

environmental conditions suggest that killifish are useful indicator species of environmental health.

CHAPTER II

SITE SELECTION AND FIELD DATA COLLECTION

2.1 Site Selection

Fundulus grandis were collected from 17 sites from Mississippi Sound to Perdido Bay (Fig. 2.1). The sites were selected to represent gradients in the environment such as salinity, urbanization, and likely local point-source inputs of contaminants (see 2.3 Physical Data Collection).

Killifish were collected in Summer 2019 (4 July – 24 August, 2019) and Spring 2020 (9 – 24 March 2020) to detect potential seasonal variations in condition that may mask or exaggerate apparent spatial patterns. While samples were collected in the Spring and Summer, some sites were not sampled in both seasons. Cedar Point (CED), Meaher Park (MEA), Wolf Bay (WOL), Fowl river (FOW), Airport Marsh (AIR), and West End Beach (WEB) were only sampled in the Summer and Arlington Park (ARL) and Weeks Bay South (WBS) only had collections in the Spring (Fig. 2.1).

Preliminary data analysis showed that *F. grandis* from saline coastal marsh sites around Dauphin Island and Mississippi Sound (AIR, CED, CFM, DISL, FTM, OYB, WEB, WOL, and WWP) had similar condition to each other in Summer 2019. Therefore, the number of saline coastal marsh sites was reduced with effort redirected to sampling likely point-source impact sites for the subsequent Spring 2020 sampling. However,

sampling at FOW and MEA were attempted in the Spring without success. ARL was sampled successfully in the Spring but not in the Summer. WBS was added to the Spring site list as a point closer to Mobile Bay than Weeks Bay North (WBN) due to a small sample size collected from WBN in the Summer.

Sample collection at Downtown Mobile sites, potential high impact sites due to their proximity to urban and local point source inputs, were attempted at least 3 times per season without success. Collection of killifish from Industrial Port Canal (IPC), ARL, and Pinto Island (PIN), encompassing some important potential impact sites, were also unsuccessful except for Arlington Park in the Spring. The inability to catch any killifish at these sites could be explained by a combination of factors. Sites in the upper Bay had very low CPUE (pers. obs.), and this could be due to salinity effects (Patterson et al. 2012), with these sites all having low salinities during the study period. Although killifish can tolerate a wide range of salinities, they show signs of physiological distress at very low salinities (Patterson et al. 2012). Additionally, the impact from the potential point sources of pollution could have decreased their density such that a greater sampling effort would be required.

One anomaly that may have affected killifish collection was a 40-year flood event in Mobile Bay just before collection began in the spring (Scheurich 2020). This large influx of fresh water could have forced killifish to move southward to avoid unfavorable conditions. While Nelson et al. (2014) shows that *F. grandis* has a limited movement range, disruptive events, like flooding, could drive fish to seek waters with a tolerable salinity level outside their usual home range (Patterson et al. 2012).

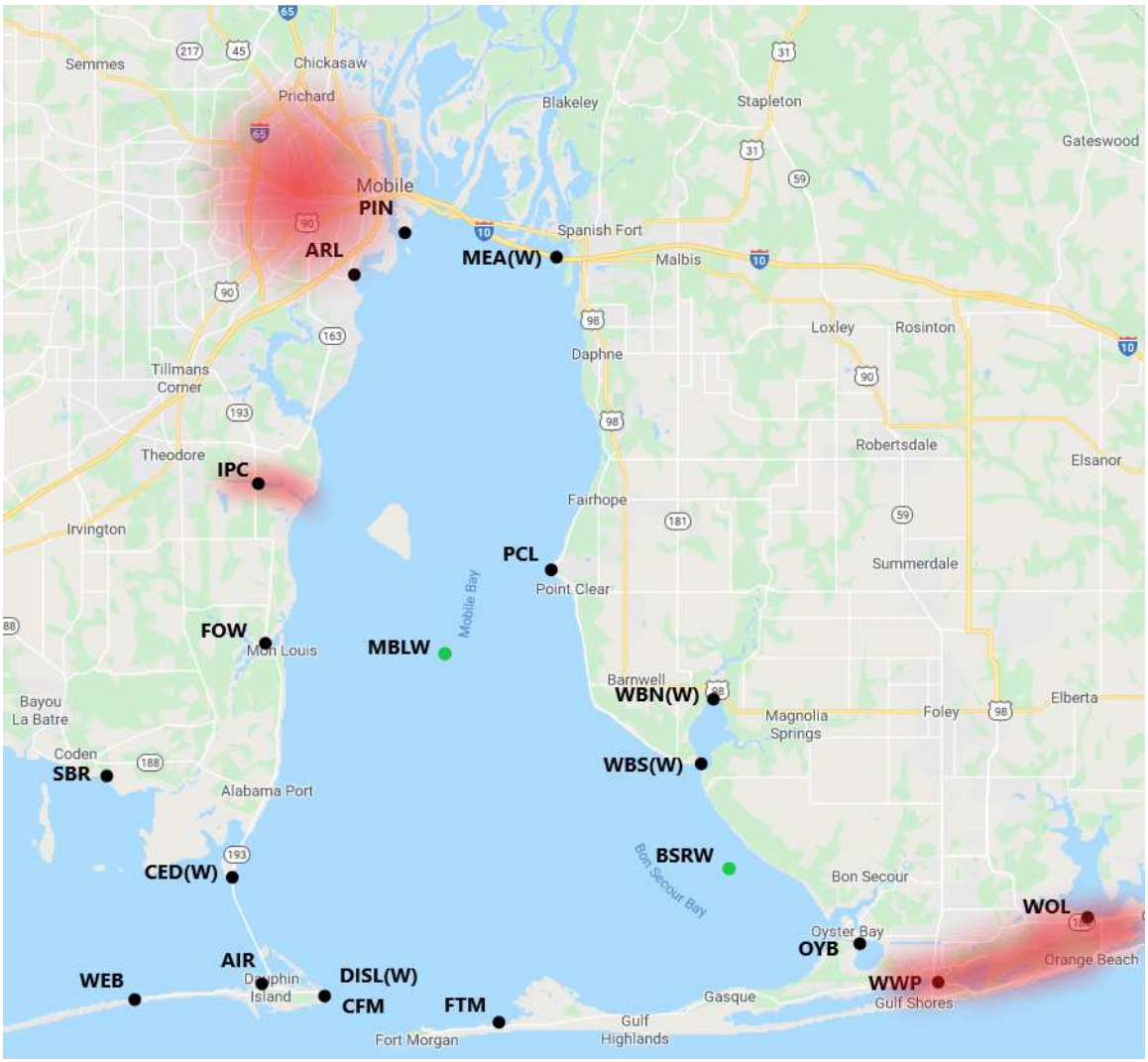

Figure 2.1. Collection sites and Weather Stations. Collection sites for Gulf Killifish in Alabama's coastal waters and Weather Stations. Potential hot spots for pollution, Downtown Mobile (upper left), Gulf Shores / Orange Beach (lower right), and Industrial Port Canal (middle left) are highlighted in red. Site acronyms are defined in Table 2.1.

Table 2.1 Acronyms for Collection Sites and Weather Stations for Figure 2.1

Site Abbreviation	Site Name	Site Type
AIR	Airport Marsh	Collection Site
ARL	Arlington Park	Collection Site
CFM	Car Ferry Marsh	Collection Site
CED	Cedar Point	Collection Site
FTM	Fort Morgan	Collection Site
FOW	Fowl River	Collection Site
IPC	Industrial Port Canal	Collection Site
MEA	Meaher Park	Collection Site
OYB	Oyster Bay	Collection Site
PIN	Pinto Island	Collection Site
PCL	Point Clear	Collection Site
SBR	Shell Belt Road	Collection Site
WWP	Wade Ward Park	Collection Site
WBN	Weeks Bay (North)	Collection Site
WBS	Weeks Bay (South)	Collection Site
WEB	West End Beach	Collection Site
WOL	Wolf Bay	Collection Site
BSRW	Bon Secour	Weather Station
CEDW	Cedar Point	Weather Station
DISLW	DISL	Weather Station
MEAW	Meaher Park	Weather Station
MBLW	Middle Bay Lighthouse	Weather Station
WBNW	Weeks Bay North	Weather Station
WBSW	Weeks Bay South	Weather Station

2.2 Field Sampling

The target sample size for each site was a minimum of 5 small and 5 large individuals, with the exact numbers in each size class dependent on the abundance of fish in the collections. Vastano et al. (2017) reported the average size at maturity for *F. grandis* of 4.9 cm total length. Once at maturity, several condition metrics are likely to change (Ch. 3). Therefore, in the present study, fish less than 4.9 cm were considered "small" while those greater than 4.9 cm were classified as "large".

Fish were collected using minnow traps baited with approximately 1-2 oz of wet cat food. Traps were deployed from the shore into submerged vegetated marsh and deployed for one to two hours before collection. A single trap deployed at FTM in the spring was left overnight after a very small sample size was observed following the 1-2 hour deployment. However, the amount of fish in the trap remained the same the next morning. Therefore 1-2 hour deployments were deemed appropriate. Fish were euthanized in an ice slurry following the approved IACUC protocol #1437888-2 and taken to the Dauphin Island Sea Lab to be stored and analyzed. A total of 226 *F. grandis* were collected. Sample size varied at each site and between seasons (Table 2.2).

2.3 Physical Data Collection

While *F. grandis* can tolerate a wide range of salinity, DO, and temperatures, extended exposure to more extreme conditions can cause stress, reduction of growth rates, and reduction in reproductive capability. For instance, near fresh water or hypersaline conditions can inhibit overall growth of *F. grandis* (Patterson et al. 2012). When *F. grandis* exists in a non-ideal salinity range, it must regulate its osmotic balance with its environment. This uses energy that otherwise could be spent on growth. Embryonic development is also adversely affected by salinity extremes, including reduced hatch percentage and rate of embryogenesis (Brown et al. 2012). If exposed to chronic hypoxic conditions, *F. grandis* displays a reduction in its capacity to metabolize

Table 2.2. Sample size of *Fundulus grandis* at each collection site in Mobile Bay, AL by season and fish size. Per Vastano et al. (2017), Small is < 4.9 cm, Large is ≥ 4.9 cm. NS indicates site was Not Sampled within that season and 0 indicates the site was sampled but no fish were collected.

	Summer 2019		**Spring 2020**	
	Small	Large	Small	Large
Airport Marsh (AIR)	1	13	NS	NS
Arlington Park (ARL)	NS	NS	0	14
Car Ferry Marsh (CFM)	3	13	0	14
Cedar Point (CED)	9	17	NS	NS
Fort Morgan (FTM)	8	19	0	4
Fowl River (FOW)	3	6	0	0
Industrial Port Canal (IPC)	0	0	0	0
Meaher Park (MEA)	0	18	0	0
Oyster Bay (OYB)	1	12	4	8
Pinto Island (PIN)	NS	NS	0	0
Point Clear (PCL)	0	4	0	4
Shell Belt Rd.	0	0	NS	NS
Ward Park (WWP)	2	3	0	7
Weeks Bay South (WBS)	NS	NS	0	11
Weeks Bay North (WBN)	4	3	2	1
West End Beach (WEB)	0	8	0	0
Wolf Bay (WOL)	4	6	0	0

carbohydrates, leading to reduced growth rates (Martinez et al. 2006). Landry et al. (2007) found that females exposed to long-term hypoxia produced significantly fewer eggs and initiated spawning later than control fish. As temperature decreases, metabolism and growth rates in fish decrease as well (Handeland et al. 2008).

Physical and environmental parameters that could affect the condition of *F. grandis* at each collection site were determined. First, the location and nature of major potential point-source inputs were determined for each site. These include the industrial port canal on the west side of Mobile Bay, which includes chemical, oil, manufacturing, and port operations industries, and heavily urbanized areas along the Gulf Shores beach

and Mobile city areas (Fig. 2.1). Salinity, dissolved oxygen, and temperature were measured at the time of sampling using a YSI ProSolo Digital Water Quality Meter. In addition to point measures at the time of sampling, long term physical data (salinity, dissolved oxygen [DO], temperature) were downloaded from Alabama's Real-Time Coastal Observing System (ARCOS) (Fig. 2.1). Since a fish's physiological condition measured as length-weight ratio, caloric content, or mass of energy reserves within the body does not change instantaneously based on immediate environmental conditions, time averages or extremes of physical conditions leading up to capture are likely to be more informative than a point measure of physical environmental conditions at the time of capture (Elliott et al. 2007). Different condition metrics are likely to respond to physical conditions at varying time scales. For example, the movement of energy reserves from lipid storage to gamete production may have relatively rapid impacts on the Hepato-Somatic Index and the Gonado-Somatic Index, but little impact on the length-weight of the fish. However, the response time for each condition metric to each physical variable is unknown, so multiple time averages were taken for each physical variable: 2 week, 1 month, 2 month, and 3 month averages for each parameter. Because DO conditions may regularly reach potentially lethal low levels in Mobile Bay during summer (Park et al. 2007), the 5^{th} percentile of minimum DO was also determined for each time interval at each site. The 5^{th} percentile of the minimum DO was used to represent the minimum DO to avoid extreme outliers and potential instrument errors.

There were two major issues with the data collected from the ARCOS system. First, there was no ARCOS station physically close enough to Arlington Park, Ward Park, or Wolf Bay to be used for analysis of physical conditions (Fig. 2.1). Second, the

Middle Bay Lighthouse station used to predict long-term physical conditions at Point Clear experienced a malfunction from 7 July 2019 through spring sampling at Point Clear. This absence of data excludes these sites (n = 44 killifish) from analysis of the relationship between physical conditions and killifish condition.

Aerial imagery from Google Earth was used to determine landscape metrics quantifying local aquatic habitat composition and terrestrial land use around each collection site. Local-scale habitat metrics quantified the proportion of vegetated marsh, open water, tide pool (open water within the marsh complex) and terrestrial land within a 100m radius from the point of collection (Fig. 2.2). The 100m radius was based on the home range of *F. grandis* measured by Nelson et al. (2014). Land-use metrics were quantified within 1km of each collection site, as the proportion of Light Urban, Heavy Urban, Industrial, Forested, or Sandy Beach (Fig. 2.2; Table 2.3).

The land use metrics classified in Table 2.3 were used as a proxy for potential land-use impacts on aquatic environmental quality. Although mapping the entire watershed of each site may provide a more realistic measure of land-use and potential runoff impacts to each sampling site, most collection sites were coastal fringing marsh sites and as such did not have clearly defined watersheds, making such an approach impractical.

Figure 2.2. Landscape metric measurements for Airport Marsh (A) at 100m for local aquatic habitat and (B) at 1km for watershed metrics. Other site maps and a table of site metrics are included in Appendix B.

Table 2.3. Classification and description of collection site areas within (A) 100m habitat and (B) 1km watershed.

A

Category	classification by terrain type	Description
Marsh	>90% vegetated marsh	vegetated marsh
Tide Pool	>90% open water surrounded by vegetated marsh	open water surrounded by marsh
Open Water	>90% open water not surrounded by vegetated marsh	open water not surrounded by marsh
Land	<5% water	land

B

Category	Classification by Land Coverage	Description
Light Urban	<50% man-made structures	some structures or roads but mostly natural
Heavy Urban	>50% man-made structures	some natural but mostly structures or roads
Industrial	presence of factories or other industrial facilities	presence of factories or other industrial facilities
Forested	0% man-made structures with at least 50% tree coverage	no structures or roads with primarily tree covered land
Sandy Beach	>50% sand along waterline	sandy beach with little to no development

CHAPTER III

VARIATION IN *FUNDULUS GRANDIS* CONDITION ACROSS COASTAL ALABAMA

3.1 Introduction

There are various measures of a fish's condition that indicate its relative mass or the distribution of energy reserves; in each instance, higher mass or more energy reserves are considered to indicate good condition. During juvenile stages in fishes, predation accounts for much of the very high mortality rates, and mortality is inversely proportional to size (Sogard 1997). Therefore, rapid growth to larger size is advantageous for fish. Once maturity is reached, fecundity is positively related to body size, often exponentially (Nunes et al. 2011), therefore, larger body size at maturity is also a fitness advantage. Having energy reserves in the form of stored lipids can therefore enhance the growth and fecundity of an individual. The various body condition metrics all relate to these factors, indicating relative body size and the distribution of stored lipids within the body.

Analyzing changes in condition metrics from an indicator species can reveal potential changes in environmental health. Wedge et al. (2015) compared the health of tidal creeks by examining condition metrics of *F. grandis* and *Poecilia latipinna*. They found that body condition metrics of both species were negatively correlated with the

extent of urbanization around the collection sites. This implies that increased urbanization may reduce environmental condition, resulting in lower condition of killifish.

Once at maturity, the condition and energy allocation of fish are likely to change as they direct more energy towards reproduction as opposed to growth. Because significant energy reserves are used in the production of gametes and spawning (Roff 1983), the recent spawning history of an individual could potentially have considerable influence on body condition. While it was not practical to histologically determine the spawning history of each individual fish examined in this study (McAdam et al. 1999), we can quantify energy reserves remaining within the body tissues and organs including the liver, gonads, and fat bodies within the abdomen.

Length-weight ratio is a simple metric that can indicate the nutritional status of a fish whereby a fish that is heavier for a given length is considered to be in better condition than lighter fish of the same length (Barton et al. 2002). However, this metric tends to be relatively coarse and insensitive to moderate variations in fish health and condition (Moles and Rice 1983). Therefore, in this study, additional condition metrics were considered.

The hepato-somatic index, HSI, is the ratio of liver mass to total body mass. Since the liver serves as a storage site for lipids (Ando et al. 1993), this index has been used to indicate a change in nutrition, condition, and reproductive state (Laurén and Wails 2018). While an increase in liver size, thus HSI, can indicate the storage of lipids, suggesting a healthy fish, it could also be caused by an exposure to toxicants, indicating a stressed or physiologically challenged fish (Laurén and Wails 2018). With an increase in

HSI potentially indicating conflicting underlying causes, HSI must be interpreted cautiously.

Similar to HSI, the gonadosomatic index (GSI) is the gonad mass as a percentage of total body mass (Anderson and Gutreuter, 1983). GSI is commonly used to determine reproductive status and periods of fish. However, the reliability of using GSI has varied based on species. GSI tends to be a more reliable index for fish species that reproduce annually (McAdam et al. 1999) compared to those that are protractive spawners that reproduce in batches over the course of a season (Rinchard and Kestemont 1996, Brewer et al. 2008). GSI is also used as an energy storage index in conjunction with other energy storage indexes (HSI etc.) to determine how much energy is being utilized for reproductive purpose (Brewer et al. 2008).

Some fish also store lipids as fat bodies directly in the abdominal cavity (Plaza et al. 2007). The percentage of the combined mass of the fat bodies to the total body mass is the lipo-somatic index (LSI). Any excess energy stored as fat indicates that the fish has satisfied all of its growth and reproductive energy needs and has excess energy to store. This would indicate the fish is in good condition and able to withstand periods of low food availability or upcoming spawning events.

This chapter aims to answer the overall hypothesis, "Does *F. grandis* body condition vary predictably among sites with varying extents of development in the catchment and along natural gradients of hydrographic properties?" This was accomplished by determining the condition of each killifish (L-W ratio, HSI, GSI, and LSI) and determining if variability in these metrics can be explained by variations in environmental condition.

3.2 Methods

Each *F. grandis* was processed to quantify each of the metrics described above. Fish were measured (total length in mm) and weighed (to the nearest 0.1g). Fish were dissected to remove and weigh the liver, gonads, and any fat bodies from within the abdominal cavity (to the nearest 0.01g). The digestive tract was removed and discarded to prevent any bait used in the traps, or other stomach contents, from affecting the caloric content of the fish, and the remainder of each fish and its respective liver, gonads, and fat bodies were then processed for use in bomb calorimetry (see Chapter 4).

Although growth rates can differ between sexes for some fish species, Vastano et al. (2017) found no difference in the growth rate between male and female *F.* grandis. Therefore, sexes were not separated, and a single growth curve was fitted to all fish sampled in the present study. Length to weight of each fish was plotted and a power-curve was fit to represent the overall length-weight relationship across AL coastal waters. The power curve was selected as the best fit out of linear, power, exponential, and polynomial models. Model selection was based on the R^2 value, examination of residual plots, the mathematical relevance of each model, and Akaike information criterion (AIC) analysis. The linear and exponential models were poor fits with low R^2, and clear lack of fit based on residuals deviating from the model fit across all TL for the linear model (Appendix Fig. C3) and at TL above 90mm for the exponential model (Appendix Fig. C4). Although both the polynomial and power curves had similarly high R^2 values (Appendix Fig. C1), the residuals of the polynomial (Appendix Fig. C5) clearly deviate from the model for smaller fish, with the polynomial model overestimating fish weight for fish smaller than about 45 mm TL, while the distribution of residuals for the power

(Appendix Fig. C2) curve indicates a good fit across the length range analyzed. Additionally, the polynomial model has no theoretical basis for describing the relationship between length and weight, while a power curve is a logical model to describe the relationship between length and weight (volume) in a three-dimensional organism. AIC analysis, in which the model with the lowest AIC value is determined to have the best fit with the least number of independent variables (Wagenmakers and Farrell 2004), also identified the power curve as the best fit model with an AIC score of -870 (Appendix Table C7). This curve was used to identify if any sites stood out from the others as having fish that were consistently heavier or lighter than predicted from the overall length-weight relationship. The residuals from the power curve (the difference between predicted and actual weight, Appendix Fig. C2) were calculated for each fish and used as an additional condition metric in analyses, termed Deviation from Predicted Weight. Negative deviations indicated fish that were lighter than predicted from the overall length-weight relationship, while positive deviations indicated fish heavier than predicted.

Analysis of covariance (ANCOVA) was performed to test if the length-weight (l-w) relationship varies among sites, specifically to test the null hypothesis that the slope or intercept of the l-w relationship does not vary among sites. The ANVOCA was performed on cube-root transformed weight values that produced a linear relationship with TL, since ANCOVA cannot be performed on non-linear relationships. The cube-root transformation is logical to linearize the relationship between length and weight.

HSI, GSI, and LSI were calculated for each fish. Within reproductively active individuals, energy stores could be moved around quickly, e.g. from liver to gonads

during gamete development (Hsiao and Meier 1989, Green 2013), adding noise to the individual metrics. Therefore, a new index was developed in the current study, the Total Energy Body Index (TEBI) which combines HSI, GSI and LSI into one metric to allow for analysis on the total amount of stored energy within these sources. The equations for each are as follows:

$$HSI = 100 \times (mass\ of\ liver\ (g) \div total\ mass\ (g))$$

$$GSI = 100 \times (mass\ of\ gonads\ (g) \div total\ mass\ (g))$$

$$LSI = 100 \times (mass\ of\ fat\ bodies\ (g) \div total\ mass\ (g))$$

$$TEBI = 100 \times ((mass\ of\ liver\ (g) + mass\ of\ gonads\ (g) + mass\ of\ fat\ bodies\ (g)) \div total\ mass\ (g))$$

GSI and LSI were not calculated if the gonads or any fat bodies were not located.

To identify the relative importance of natural gradients and potential pollution impacts on variability in killifish condition, classification and regression trees (CART) were employed (Loh 2014). CART analysis successively splits the data into increasingly homogeneous groups, by minimizing the residual sums of squares for each split, analogous to least squares regression (De'ath and Fabricius 2000). They provide a powerful means of explaining variability in data, and can include combinations of continuous (e.g. salinity, proportion of urbanized catchment) and categorical explanatory variables (e.g. season), and are robust in their ability to analyze unbalanced data sets (Loh 2014). CARTs were used to determine which physical, habitat, and catchment variables explained variation in killifish condition.

A series of univariate CARTs were run to explain variability in each of the condition metrics. For each CART, the response variable was one of the condition

metrics (Deviation from Predicted Weight, HSI, GSI, LSI, TEBI) and explanatory variables were: killifish total length; season (Summer, Spring); % composition of each habitat category within 100 m of the collection site, and land-use categories within 1 km (Ch. 2). Since not all sites included physical parameters due to distance from an ARCOS station (Ch. 2), a second set of CARTs were run for each condition metric that only included the subset of sites that also had physical parameters included. The derivation of multiple overlapping timeframes of physical variables from the ARCOS data (Ch. 2.3) produced a set of non-independent explanatory physical variables. Rather than including all these in the CART models, a correlation matrix was run to identify which time scales (2wk, 1 mo, 2 mo, 3 mo) for each environmental parameter (salinity, temp, DO) correlated most strongly with the various condition metrics of the killifish. As expected, the different time frames within each physical parameter tended to be highly correlated with each other, however, no physical parameters were highly correlated ($r < 0.50$) with any of the condition metrics (Table. 3.1). This indicates that most of the variation in any given condition metric cannot be attributed to any particular physical parameter but is most likely a combined effect of multiple factors. The physical parameter-time frame combinations with the highest correlation coefficient with the condition metrics were 1-month mean salinity, 1-month mean DO, 2-month minimum DO, and 2-month mean temperature (Table 3.1), and these were used as explanatory variables in subsequent analyses. Only CART models that produced a significant fit are presented (Table 3.2). The remaining models did not produce significant fits, i.e. they did not explain any variability in the relevant condition metric.

Table 3.1. Correlation matrix identifying the timeframes for each water quality parameter from the ARCOS stations that are most strongly correlated with the condition metrics described in Chapter III. The R value for the strongest correlation between each water quality parameter and timeframe is highlighted in green, and these parameter-timeframes were used in subsequent analyses modeling variation in killifish condition. Salinity = mean salinity (psu), DO = mean dissolved oxygen (mg/L), Min DO = minimum dissolved oxygen (mg/L), Temp = mean temperature (°C)

Water Quality Parameter	Timeframe	Condition Metrics (described in Ch.3)		
		Difference from Expected Weight	HS Index	TEB Index
Salinity	*2 weeks*	0.4664	-0.0993	0.0531
Salinity	*1 month*	0.4669	-0.1116	0.0371
Salinity	*2 months*	0.4059	-0.1356	-0.0151
Salinity	*3 months*	0.3042	-0.1098	-0.0867
DO	*2 weeks*	-0.2585	-0.0668	-0.2555
DO	*1 month*	-0.2873	-0.0167	-0.2003
DO	*2 months*	-0.2787	0.0395	-0.1469
DO	*3 months*	-0.2418	0.089	-0.0845
Min DO	*2 weeks*	-0.3681	0.0087	-0.1951
Min DO	*1 month*	-0.4095	-0.0304	-0.1621
Min DO	*2 months*	-0.4195	-0.001	-0.1566
Min DO	*3 months*	-0.4156	0.0402	-0.1059
Temp	*2 weeks*	0.1281	-0.0472	0.0874
Temp	*1 month*	0.302	-0.1763	0.1082
Temp	*2 months*	0.3538	-0.164	0.0918
Temp	*3 months*	0.3273	-0.1696	0.0953

3.3 Results

Fundulus grandis collected from Meaher Park were consistently lighter than predicted by the overall length-weight model for all fish (Fig. 3.1). Arlington Park, Meaher Park, Point Clear, and Fowl River had significantly lower intercepts (ANCOVA $p<0.001$, Table 3.3). This indicates that *F. grandis* from Arlington Park, Meaher Park, Fowl River, and Point Clear were significantly lighter for their size than fish collected at other sites. These sites were the northern-most, upper Bay sites from which fish were successfully collected (Fig. 2.1, Table 2.1).

Overall, season explained a large amount of variation in Deviation from Predicted Weight, with fish collected in summer being heavier than those collected in the spring (Fig. 3.2). Among fish collected in the summer, *F. grandis* larger than 74 mm TL were heavier than predicted (Fig. 3.2). Among the *F. grandis* smaller than 74 mm in length collected in the Summer, those from sites whose watershed contained more than 54% marsh were lighter than those from sites with less marsh. When analyzing the subset of data that includes sites that had physical data, salinity was identified as a key predictor of deviation from predicted weight as well. The 49 fish from sites with 1-month average salinity less than 2.02 were lighter than predicted from the overall l-w relationship (Fig. 3.3). Among the higher salinity sites, the 90 fish smaller than 68 mm TL were close to their predicted weights, while the 35 individuals over 68 mm TL tended to be heavier than expected for their length (Fig. 3.3).

Table 3.2. Description of Classification and Regression Tree analyses that provided a significant fit explaining variability in *Fundulus grandis* condition among sites in Alabama's coastal waters. DO = Dissolved Oxygen.

Analysis	Condition Metric	Included Explanatory Variables
Fig. 3.3	Deviation from Predicted Weight – All sites	Season Collection site composition (marsh, tide pool, open water, or land) Watershed composition (Light urban, Heavy Urban, Industrial, Forested, Sandy Beach, Open Water, or Marsh)
Fig. 3.4	Deviation from Predicted Weight – subset of sites with long-term physical data	Season Collection site composition (marsh, tide pool, open water, or land) Watershed composition (Light urban, Heavy Urban, Industrial, Forested, Sandy Beach, Open Water, or Marsh) Salinity (1 month average) DO (1 month average) Minimum DO (2 month minimum) Temperature (2 month average)
Fig. 3.5	Total Energy Bodies Index – All sites	Season Collection site composition (marsh, tide pool, open water, or land) Watershed composition (Light urban, Heavy Urban, Industrial, Forested, Sandy Beach, Open Water, or Marsh)
Fig. 3.6	Total Energy Bodies Index – subset of sites with long term physical data	Season Collection site composition (marsh, tide pool, open water, or land) Watershed composition (Light urban, Heavy Urban, Industrial, Forested, Sandy Beach, Open Water, or Marsh) Salinity (1 month average) DO (1 month average) Minimum DO (2 month minimum) Temperature (2 month average)

When the condition metrics of *F. grandis* were plotted against each other to explore potential interactions between condition metrics, only HSI vs TEBI and GSI vs TEBI had a significant trend (Appendix Fig. D1 and Fig. D2). These relationships were

to be expected since HSI and GSI are components of TEBI. All other combinations of TL, HSI, GSI, LSI, and TEBI showed no apparent relationships (Appendix D).

None of the CART models for HSI, GSI or LSI were significant, however, some variation in TEBI was explained by landscape metrics (Fig. 3.5). *F. grandis* TEBI was higher in sites whose watershed within 1 km was greater than 56% forested. At sites with more urbanization, *F. grandis* had lower TEBI. When analyzing the subset of data that included the physical data, *F. grandis* had lower TEBI in habitats with more than 33% open water (Fig. 3.6).

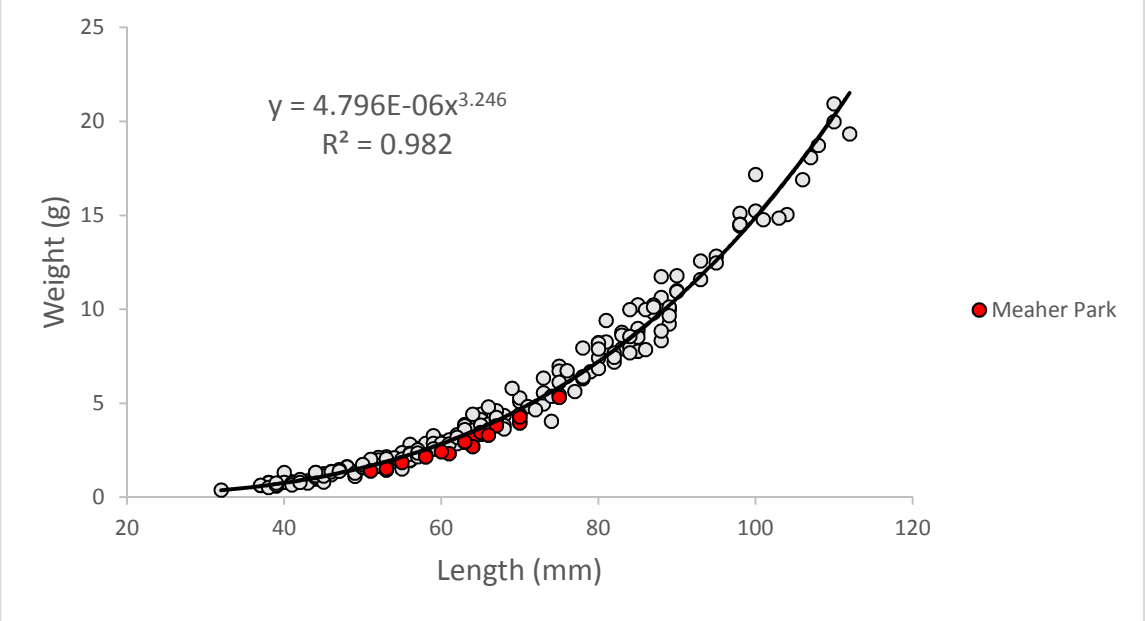

Figure 3.1. Length - weight relationship for killifish (*Fundulus grandis*) (n = 234) from coastal waters of Alabama. Meaher Park (red symbols) is highlighted as the most extreme site where all individuals were lighter than predicted by the power curve.

Table 3.3. ANCOVA of length to weight ratio shows that both cube root transformed weight and sample site are significant cofactors of total length of *Fundulus grandis*.

ANCOVA

Source of Variation	*df*	*SS*	*MS*	*F*	*Significance F*
Transformed Weight	1	28.90	28.90	11204.77	1.53E-05
Sample Site	13	0.23	0.02	6.72	1.03E-10
Residual	209	0.54	0.003		
Total	223	48.27			

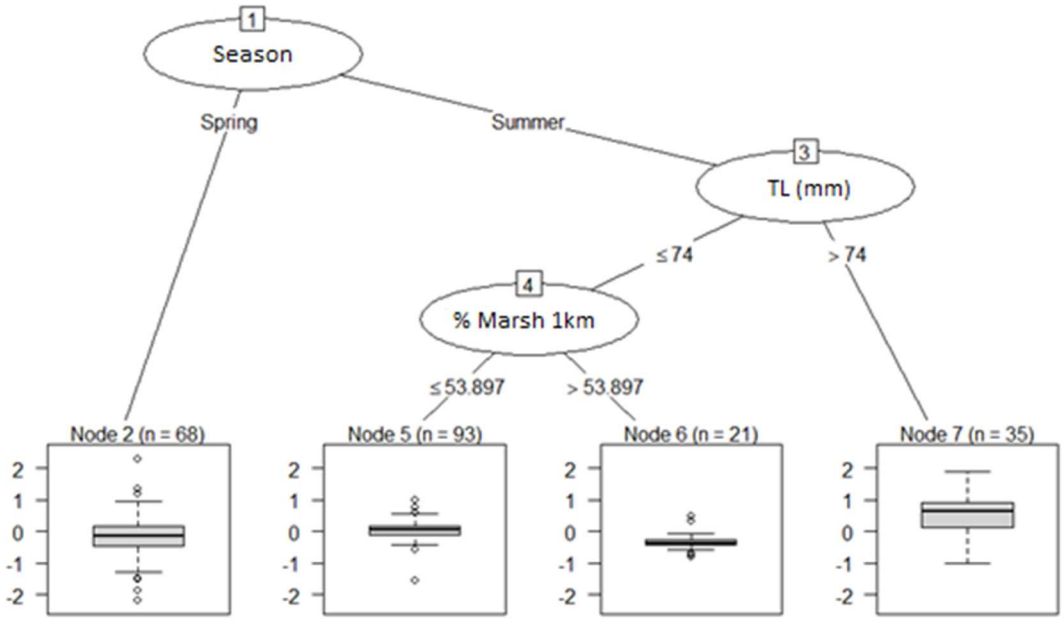

Figure 3.2. CART analysis explaining variation in killifish condition in Alabama's coastal waters, based on Deviation from Predicted Weight (Fig. 3.1). Explanatory variables were fish TL, Season, seascape habitat composition metrics (Table 2.4A), and catchment land use metrics (Table 2.4B). Ovals indicate the explanatory variable forming each split in the final model (Nodes 1, 3, 4). Text on the branches leading from each split indicate the categories or values at which each split formed. Terminal nodes (Nodes 2, 5, 6, 7) indicate the sample size (n = number of fish) in each terminal node, and box plots show the distribution of Deviation from Predicted Weight values (g) of those fish.

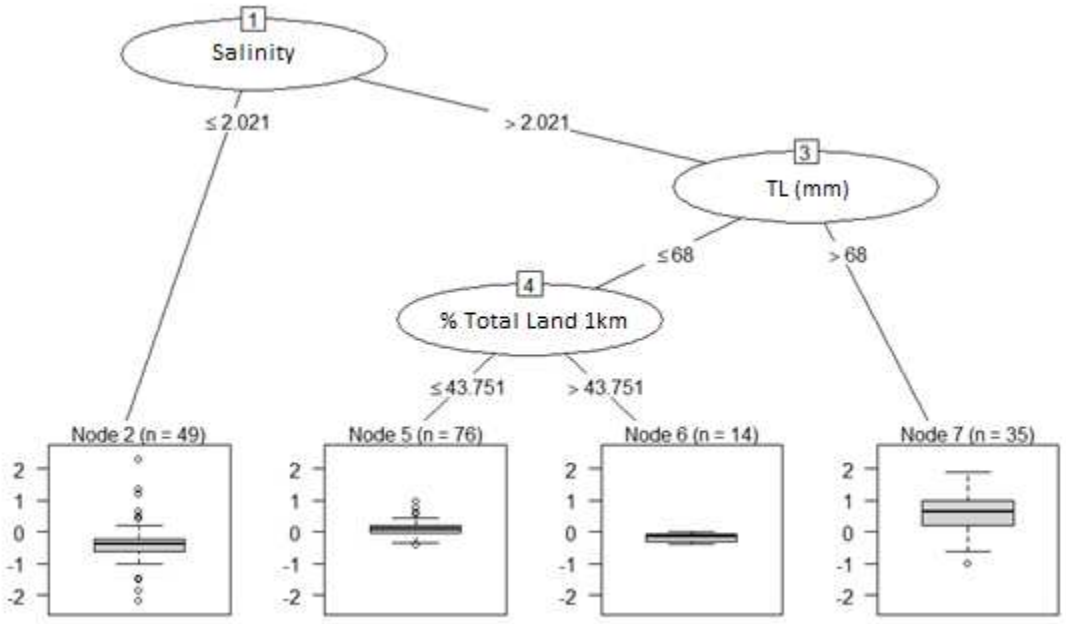

Figure 3.3. CART analysis explaining variation in killifish condition in Alabama's coastal waters, based on deviation from predicted weight (Fig. 3.1) using only the subset of sites for which long term physical data were available. Explanatory variables were fish TL, Season, seascape habitat composition metrics (Table 2.4A), catchment land use metrics (Table 2.4B), and water quality metrics (Table 3.1). Ovals indicate the explanatory variable forming each split in the final model (Nodes 1, 3, 4). Text on the branches leading from each split indicate the categories or values at which each split formed. Terminal nodes (Nodes 2, 5, 6, 7) indicate the sample size (n = number of fish) in each terminal node, and box plots show the distribution of Deviation from Predicted Weight values (g) of those fish.

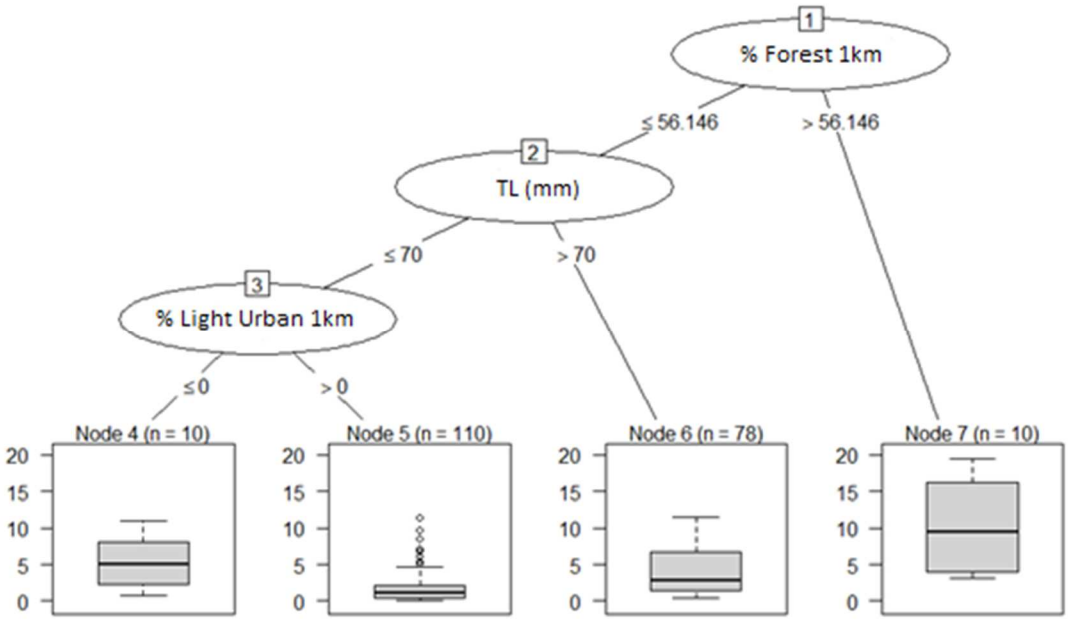

Figure 3.4. CART analysis explaining variation in killifish condition in Alabama's coastal waters, based on Total Energy Bodies Index. Explanatory variables were fish TL, Season, seascape habitat composition metrics (Table 2.4A), and catchment land use metrics (Table 2.4B). Ovals indicate the explanatory variable forming each split in the final model (Nodes 1, 2, 3). Text on the branches leading from each split indicate the categories or values at which each split formed. Terminal nodes (Nodes 4, 5, 6, 7) indicate the sample size (n = number of fish) in each terminal node, and box plots show the distribution of Total Energy Bodies Index values of those fish.

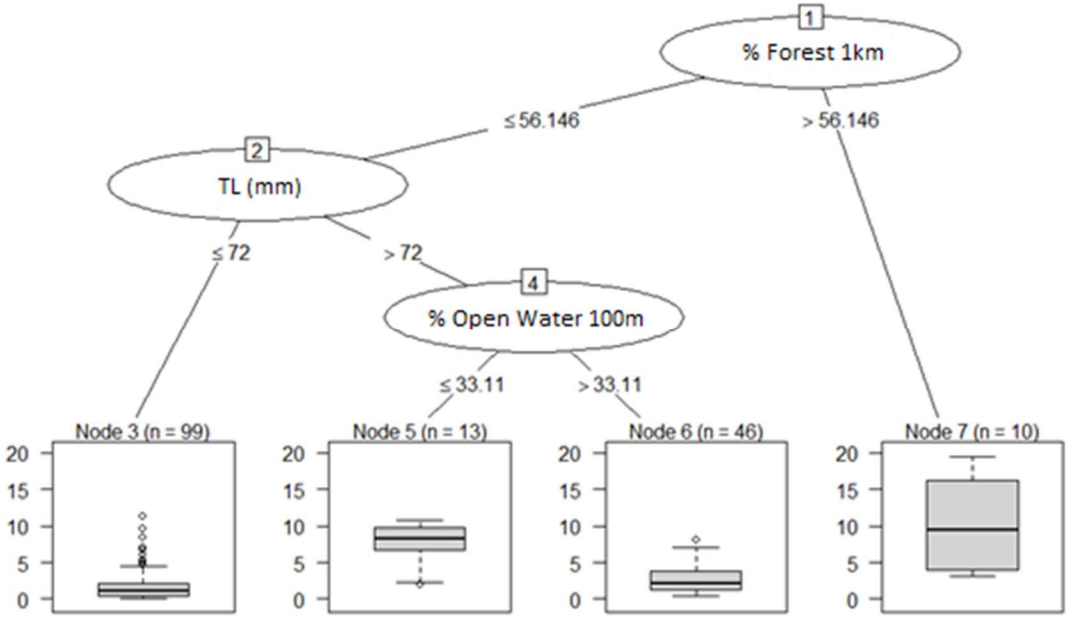

Figure 3.5. CART analysis explaining variation in killifish condition in Alabama's coastal waters, based on Total Energy Bodies Index, using only the subset of sites for which long term physical data were available. Explanatory variables were fish TL, Season, seascape habitat composition metrics (Table 2.4A), catchment land use metrics (Table 2.4B), and water quality metrics (Table 3.1). Ovals indicate the explanatory variable forming each split in the final model (Nodes 1, 2, 4). Text on the branches leading from each split indicate the categories or values at which each split formed. Terminal nodes (Nodes 3, 5, 6, 7) indicate the sample size (n = number of fish) in each terminal node, and box plots show the distribution of Total Energy Bodies Index values (g) of those fish.

3.4 Discussion

Fundulus grandis condition metrics did vary predictably among sites with varying extents of development in the catchment, varying habitat configurations in the local seascape, and along natural salinity gradients. ANCOVA identified the four upper bay sites as having fish that were significantly lighter than predicted by the overall length-weight model, while CART analysis found salinity explained significant amount of the variability in deviation from predicted weight. Sites with lower levels of urbanization in the surrounding landscape tended to have heavier/better condition fish, in agreement with the findings of Wedge et al. (2015), while seascapes, on the catchment scale, with more marsh, i.e. less open water habitat, tended to have lighter fish (Fig. 3.2).

Fundulus grandis collected from the upper bay were significantly lighter for their length than was predicted by the overall l-w model (Fig. 3.1, Table 3.1). In addition, the CART analysis that included physical data indicated that salinity was a significant driver of the l-w condition metric (Fig. 3.3). This decrease in l-w ratio in low salinity environments could be due to more energy being used to maintain osmotic balance in the fresher water, thus removing energy that could otherwise be used for growth (Patterson et al. 2012).

Indirect effects of salinity could also drive the observed patterns in body condition. Rozas and Minello (2011) demonstrated that salinity can indirectly impact growth rates of penaeid shrimps by limiting their prey. They showed that giving additional food to the penaeid shrimps in the low salinity conditions resulted in similar growth rates to shrimp held in high salinity conditions. *F. grandis* typically prey on crabs, amphipods, tanaids, and hydrobiids (Rozas and LaSalle 1990) which are mostly found in

the intertidal or subtidal zones within the salt marsh, where *F. grandis* prefer to feed. Low salinity can dramatically reduce survival, species richness, and species abundance within these groups. Hyale crassicornis, an amphipod, shows a dramatic drop in survival rates when exposed to brackish conditions for just 96 hours (Tsoi et al. 2005). Most tanaidaceans occur in marine habitats and only occur temporarily in non-marine habitats (Jaume and Boxshall 2007). Hydrobiidae species richness and abundance decreases with decreacing salinity (Gérard et al. 2003). These species are likely in less abundance or absent in fresher waters, reducing the available prey for *F. grandis* in these low salinity sites.

While direct physiological or indirect food-web effects of salinity on *F. grandis* condition are both plausible explanations for the observed patterns, it is possible that some other correlated or confounded factor may be driving these patterns. For instance, water slows when it enters the bay and thus contaminants carried from the large catchment or local sources could settle out and remain in the upper bay areas. This potential long-term exposure to a higher concentration of contaminants could reduce *F. grandis* body condition (Barton et al. 2002, Laurén and Wails 2018), leading to a reduction in l-w ratio seen in the analysis. However, based on existing evidence, salinity seems to be a driving factor for the reduction in l-w ratio observed.

Large *F. grandis*, >74mm, collected in the summer tended to be heavier for their length than predicted (Fig. 3.2). This potential seasonal trend could be due to limited food availability over the winter (Van Dolah 1978) requiring time throughout the spring and summer to build up body condition. Another possibility is that due to low numbers of very large *F. grandis* collected, the predictive model might not be accurate at large TL.

When analyzing HSI in this study, no significant trends emerged. Since a large HSI could indicate a good condition due to storage of lipids or a poor condition due to toxicant exposure (Laurén and Wails 2018) and there were no significant trends, no conclusion could be drawn from HSI in this study. Future studies could chemically analyze the liver composition of each fish to determine lipid levels (Yan et al. 2015). This could identify whether a fish with a high HSI is healthy and storing lipids or has been attempting to detoxify contaminants that it has been exposed to. In the case of the latter, the collection site that the fish was collected from could then be marked as a potential hot spot of pollution.

TEBI was developed as a way to overcome variability in individual mass indexes (HSI, GSI, and LSI) due to energy being shunted around during gamete development or rapid growth. Analysis of TEBI revealed a negative relationship between urbanization in the watershed and energy stores of *F. grandis* (Fig. 3.5). This supports the findings of Wedge et al. (2015) who concluded that *F. grandis* condition is negatively impacted by urbanization within the watershed. Urbanization can increase runoff into the estuary system. This runoff can carry with it excess nutrients (Arismendez et al. 2009; Yang 2012), heavy metals (Sanger et al. 1999, Holland et al. 2004), pesticides (Sanger et al. 2004), and other pollutants (Van Dolah et al. 2008) that can impact the condition of the estuary and the condition of the species that live there.

The spawning behavior of *F. grandis* likely added variability to the condition metrics quantified in the current study. They show semilunar spawning patterns through a protracted spawning season spanning from March to October (Hsiao and Meier 1989, Green 2013). Throughout this season, reproductively mature individuals may undergo

rapid changes in body condition as they accumulate energy stores, undergo gametogenesis, develop their gametes, and then release them (Barber and Blake 1981). In the current study, some mature females had ovaries that contributed up to almost 20 % of their total body mass (Fig. 3.5b), representing a significant amount of energy being released from the body at spawning. Gonad size was highly variable (Fig. 3.5b) suggesting that the timing of spawning is not highly synchronized across all individuals in the population. So, while sampling was conducted across all sites each season within the shortest feasible timeframe, different individuals, even from single sampling sites, were likely at different stages of the reproductive cycle. Some individuals collected may have just spawned, while others may be close to spawning. This pattern of spawning would add variability to the condition metrics quantified in this study. Planning collection times to avoid peak spawning periods and utilizing additional personnel to reduce the sampling window each season, could reduce this variability. Despite these potential issues, this study still found patterns of variation in killifish condition among sites that could be explained by catchment, seascape and physical variables.

Fundulus grandis body condition did vary across Mobile Bay and the Alabama coastal region and some of this variation can be explained with aspects of local site condition including catchment land use and local habitat configuration. This combined with the high site fidelity of *F. grandis* (Nelson et al. 2014) lends support to the use of *F. grandis* as an indicator species for local environmental health.

CHAPTER IV

THE USE OF CALORIC CONTENT AS AN INDICATOR OF BODY CONDITION FOR *FUNDULUS GRANDIS* IN ALABAMA'S COASTAL WATERS

4.1 Introduction

The previous chapter used morphometric indices of condition, including length-weight relationships, and indices of the mass of specific body parts (liver, gonads, fat bodies), to examine patterns and drivers of killifish condition across Alabama's coastal waters. While coarse morphometric indices like L-W ratio tend to be relatively insensitive to shifts in environmental condition (Moles and Rice 1983), killifish from the low salinity upper-bay sites were found to be lighter than predicted from the overall length-weight relationship (Ch. 3). Mass indices (HSI, GSI, and LSI) potentially provide a more sensitive measure of energy stores within specific organs or compartments (Plaza et al. 2007, Brewer et al. 2008, Laurén and Wails 2018). However, no clear results were drawn from these individual metrics, while the Total Energy Body Index (TEBI) developed in the current study, which combines HSI, GSI, and LSI into one index, did produce significant models explaining variation in killifish condition. These findings suggest that for killifish, condition indices that account for total energy reserves within the whole body may be more useful than indices based on the mass of individual body components.

The TEBI metric was developed to try to overcome variation in individual mass metrics due to the rapid movement of energy reserves among body compartments due to, for example, repeat spawning throughout the study period. While TEBI gives a more complete index of stored energy, it doesn't account for energy reserves located elsewhere in the body, such as within the musculature (Arrington et al. 2006).

Measuring the caloric content, the amount of energy per unit of weight, of whole homogenized fish can account for energy stored in different forms (e.g. lipids, proteins, carbohydrates) and regardless of where it is located in the fish (Moles and Rice 1983). As such, caloric content may be a more appropriate metric of body condition in situations where energy is likely to be shifting throughout the body, e.g. during spawning season for species that spawn repeatedly (Hsiao and Meier 1989, Green 2013).

Caloric content has been used to compare fish condition in different sites (Vondracek et al. 1996) as well as exposure to different levels of toxicants (Moles and Rice 1983). Exposure to toxicants can decrease overall caloric density, because energy spent eliminating or detoxifying the toxicant is diverted from growth or reproduction (Moles and Rice 1983). Moles and Rice (1983) found that juvenile pink salmon, *Oncorhynchus gorbuscha*, exposed to sub-lethal concentrations of naphthalene or crude oil showed decreased caloric content after a 40-day exposure period. Wedge et al. (2015) found that *F. grandis* from tidal creeks with more urbanized catchments had significantly lower caloric content than those from creeks with more natural catchments. Thus, a low caloric content could indicate poor condition due to low energy reserves, exposure to toxicants, or other impacts to general ecosystem health. The aim of this chapter was to

assess the use of caloric content as an alternate metric for examining patterns in killifish condition in relation to environmental variables.

4.2 Methods

4.2.1 Sample preparation

Each sample killifish had its digestive tract removed so that no bait or stomach contents would skew the results of the caloric content of the fish. Each sample was dried in a drying oven at 60°C for 72 hours to ensure no moisture remained in the sample. Samples were then homogenized using a coffee grinder and stored in a sealed vial at room temperature until ready for bomb calorimetry.

4.2.2. Calculating caloric content

Caloric content was measured using a bomb calorimeter. Once each day, before running any samples, a calibration using benzoic acid was performed. Benzoic acid has a known heat of combustion of 6317.9 cal/g (Parr Instrument Company, 2008). This was used to then calculate the cal/g of each sample by using the following formula (Parr Instrument Company, 2008).

$$-q = ((t \times C_{y,cal}) - e) \div m$$

$C_{y,cal}$ is the calorimeter constant which is calculated using the benzoic acid calibration run. The variables q and m are the calories per gram and mass in grams of the sample, respectively. The variables e and t are calculated using the following equations.

$$e = 2.3\, cal/cm \times l$$

$$t = t_c - t_a - r_1(b - a) - r_2(c - b)$$

The variable *l* is the length of the fuse wire in cm used in the run. The variables *a*, *b*, and *c* are the time of firing, the time when the temperature reaches 60% of the total rise, and the time when the temperature becomes constant after the rise in minutes, respectively. The variables t_a and t_c are the temperatures at the respective times *a* and *c* in °C. Lastly, the variables r_1 and r_2 are the rates of temperature change (°C/min) due to ambient conditions during the 5 minutes leading up to time *a* and the 5 minutes after time *c* respectively.

4.2.3 Bomb Calorimeters

Two separate oxygen bomb calorimeters were used due to complications in facility access arising from the COVID-19 pandemic. An initial set of Summer 2019 samples (n = 21) were analyzed using a Parr Model 1341 Oxygen Bomb Calorimeter in the Chemistry Department at USA. This instrument requires 1 g pellets of the dried, homogenized tissue. This meant that the majority of dried tissue from each sample was consumed in a single analysis on this instrument. When a killifish was less than 1-gram dried weight, multiple similar size killifish from the same site were combined to achieve the required 1 g sample. The required amount of sample was measured and pressed into a pellet using a pellet press. The bomb was assembled with the pellet inside and was charged to 20 atmospheres of O_2. The bomb was then suspended and submerged in 2L of cold water within an insulated container and connected to the igniter via 2 leads. Once the insulated container was sealed, a thermometer was inserted through an access port. The temperature of the water was recorded every 30 seconds for 5 minutes before ignition, during the burn, and for 5 minutes after the temperature leveled off after the burn. Each

run, including the reset for the next run, lasted approximately 40-45 minutes. Because of the relatively long timeframes to analyze each sample, these analyses were primarily run after-hours when the lab was not being used for other purposes.

After-hours access to the lab space was restricted due to covid, so the remaining Summer 2019 and Spring 2020 samples (n = 131) were run using a Parr Model 6725 Semi-Micro Bomb Calorimeter at the Dauphin Island Sea Lab. This instrument required 0.1 g pellets of dried, homogenized killifish. The ability to analyze much smaller amounts of tissue allowed for replicate analyses to be run on individual samples (see below). The semi-micro calorimeter also had the advantage of automatically recording temperature and calculating caloric content. Since the instrument automatically recorded temperature, preparation of subsequent samples could be performed while the instrument processed a sample. This allowed for near continuous runs, which lasted approximately 15 minutes.

The semi-micro bomb calorimeter was in storage for an unknown amount of time before use in this study. The initial test run revealed that the port for allowing the combustion chamber to be filled with oxygen was rusted shut. A replacement valve was ordered and attached a week later. The second test run revealed that the rust extended past initial estimates. The current used to trigger ignition of the fuse wire was prevented from reaching the interior of the combustion chamber by the rust as well. Subsequently, all parts that interfaced between the combustion chamber and the outside environment were replaced.

Because of the large amount of tissue required by the oxygen bomb calorimeter, almost all available tissue from the initial 21 samples was used. Tissue remained from 3 large killifish that weighed more than 1 gram when dried. These three samples were used

to test the comparability between the oxygen bomb calorimeter and the semi-micro bomb calorimeter. There was sufficient tissue in these samples to allow for three replicate analyses on the semi-micro bomb for each fish. These analyses allowed for comparison of caloric content values between the two instruments, and an assessment of the precision of the values from the semi-micro bomb.

4.2.4 Analysis

As in Chapter III, univariate CARTs were used to examine patterns of variability in killifish condition among sites. In this instance, caloric content was the response variable, and habitat composition, watershed composition, and physical parameters of the collection site were the explanatory variables. Because of a lack of consistency in caloric values derived from the two instruments (see below), data form the two instruments were analyzed separately.

4.3 Results

The three killifish analyzed on both bomb calorimeters used in this study revealed large and inconsistent differences in measured caloric content between the two instruments (Table 4.1). The semi-micro bomb estimated the caloric content of fish NL001 at 7% higher than the oxygen bomb calorimeter, fish NL002 at 24% lower, and the three replicates on the semi-micro bomb for NL003 spanned the value from the oxygen bomb calorimeter, with an average difference of 13% lower (Table 4.1). The large and inconsistent differences in caloric content values obtained from the two instruments indicated that data from the two could not be directly compared.

Table 4.1. Comparison of killifish caloric content values from an oxygen bomb calorimeter and a semi-micro bomb calorimeter (pre-fix), and the precision of values from the semi-micro bomb. The Oxygen Bomb required 1g of tissue per sample, hence only one replicate was possible for each sample. The micro bomb required 0.1 g of tissue, allowing for three replicates per sample to assess precision and compare with the oxygen bomb. The average of the 3 micro bomb replicates is presented, with the CV (coefficient of variation) in parenthesis. "Difference" is the average semi-micro bomb minus rep 1 of the oxygen bomb. Values reported are in cal/g

	Oxygen Bomb	Semi-micro Bomb				
Sample	Rep 1 (cal/g)	Rep 1 (cal/g)	Rep 2 (cal/g)	Rep 3 (cal/g)	Ave (CV) (cal/g)	Difference
NL 001	3847	4148	4193	4097	4146 (1.16%)	+298 (+7%)
NL 002	4434	3556	3443	3713	3571 (3.80%)	-863 (-24%)
NL 003	3650	2859	3782	3034	3225 (15.20%)	-424 (-13%)

The lack of precision in the three replicates for each of the three fish on the semi-micro bomb (Table 4.1) indicated unreliable caloric content values were being obtained from this instrument. The source of this large variation was suspected to be related to insufficient homogenization of the tissue samples.

The relatively coarse homogenization of whole fish using the coffee grinder (Fig. 4.1a) was sufficient for use in the oxygen bomb calorimeter where 1g of each sample was used, because each sample pellet analyzed comprised most of an individual large fish, or multiple small individuals. However, because the semi-micro bomb calorimeter only required a sample of 0.1g, each pellet represented a small part of an individual fish. Incomplete homogenization meant that replicate 0.1g pellets from the same individual fish may have been comprised of quite different body components, e.g. muscle or liver tissue Vs fish scales, with very different caloric contents. To overcome this, the samples with sufficient tissue remaining (n = 43) were further homogenized by manual grinding

using a mortar and pestle. The ground material was then sifted through a 500-micron sieve which separated fish scales (Fig. 4.1b) from other tissues (Fig. 4.1c). The material captured on the sieve was returned to the mortar and pestle for additional grinding, and re-sieved to ensure maximum separation of scales from other tissues.

After the more rigorous homogenization process, the variability between replicate samples was greatly reduced (Table 4.2). However, the number of samples with enough material left to be run though the semi-microscale bomb calorimeter was also greatly reduced (n = 43) after the scrapped bomb calorimeter runs and the homogenization process. CART analysis of caloric content of the remaining post-fix samples produced no significant models, meaning none of the variation in caloric content could be explained by site-specific characteristics.

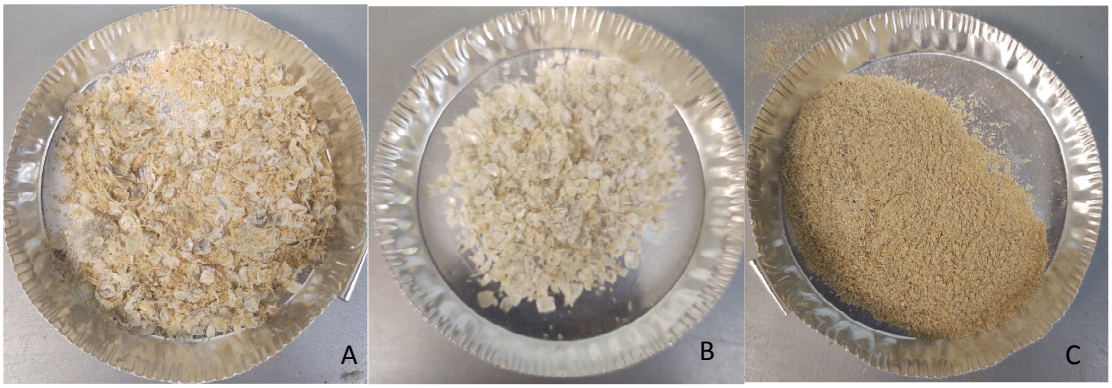

Figure 4.1. Stages of killifish homogenization. A) Homogenization with a coffee grinder. B) Removal of scales after mortar and pestle using a sieve. C) Homogenization after coffee grinder, mortar and pestle, and sieve.

Table 4.2. Bomb calorimetry statistics. Average is reported in cal/g. A total of 162 unique samples were analyzed. There were 30 samples that overlapped between the pre and post fix and 3 samples that overlapped between the oxygen bomb calorimeter and the semi-micro (pre-fix).

	Count	Average	SD
Bomb calorimerter	21	4146	258
Semi-micro (pre-fix)	131	4406	2884
Semi-micro (post-fix)	43	4658	294

4.4 Discussion

Caloric content has shown promise in other studies for revealing patterns in killifish condition among sites (Wedge et al. 2015). Conceptually, this study shares many similarities with Wedge et al. (2015), by comparing killifish condition among sites with different levels of urbanization in the catchment. However, despite significant effort and resources, our models were unable to explain any variability in killifish caloric content among sites.

The methods used for the caloric content determination are a likely source of error that future researchers can improve upon. The error introduced by inadequate homogenization of fish samples for use in the semi-micro bomb appeared to have been overcome by the more thorough homogenization process employed. However, insufficient samples remained to examine spatial patterns in condition. In addition, because the caloric content values obtained from the calorimeter are sensitive to the precise mass of material combusted (equations in 4.2.2.), any moisture absorbing into the sample between drying and combustion would introduce error into the value obtained.

Therefore, for future studies wishing to quantify the caloric content of killifish or other fish species, I recommend the following:

1) Fish should be thoroughly homogenized using the multi-step process described above.
2) Homogenized samples should be redried in the drying oven prior to analysis in the bomb calorimeter to remove any moisture that may have been introduced during homogenization or storage.
3) An initial set of samples should be analyzed in triplicate before running the main set of samples to ensure the method is producing repeatable and comparable results.

Even when these methodological challenges are overcome, the patterns of killifish spawning may complicate use of caloric content as a condition metric. One potential explanation for caloric content showing no significant results could be that the energy loss from spawning is greater than the variability between sites. Fish will typically have higher energy reserves before spawning and have little to no energy reserves after spawning (Roff 1983, McBride et al. 2015). Also, spawning is not tightly synchronized among individuals across Mobile Bay and coastal Alabama (Hsiao and Meier 1989, Green 2013), therefore, even sampling from each site on the same day would likely produce individuals at different stages within the spawning cycle.

There are a couple of methods that could potentially overcome these difficulties. First, sampling time could be targeted for the midpoint in the spawning cycle, during the neap tide phase (Hsiao and Meier 1989). This would reduce the possibility of collecting *F. grandis* that have recently spawned along with those that still have their eggs, thus

reducing the variability of stored energy between fish. Another method would be to significantly increase sampling size. This would allow for analysis of a subset of individuals determined to be at similar stages of the reproductive cycle. Categorization of an individual's reproductive stage could be done through macroscopic examination of the gonads (Ferreri et al. 2009). A potential drawback to this method is that it requires a much greater sample size, which potentially poses an issue for collection sites where few or no *F. grandis* were able to be collected, including some of the potential hotspots that were targeted in this study. Despite this and based on the findings of Wedge et al. (2015), caloric content is worth investigating further.

CHAPTER V

CONCLUSION

Various *F. grandis* condition metrics did vary across Mobile Bay and Alabama's coastal waters. Some of this variability can be explained by catchment land use, local habitat, season, and salinity. This suggests that *F. grandis* is a useful indicator species for environmental health.

One of the main drivers of variation in *F. grandis* condition identified in this study was salinity. Low salinity could be increasing the energy need of *F. grandi*s for osmoregulation (Patterson et al. 2012) or could be reducing available food for *F. grandis* (Rozas and Minello 2011). Another possibility is that suspended contaminants settle out once the water slows at the entrance to the bay (Stewart 2020). These contaminants could then accumulate in the sediments of the upper bay and degrade the environment, resulting in the reduced body condition of *F. grandis* seen in the low salinity, upper bay sites. While this could confound the result of salinity being an important driver of *F. grandis* condition, many previous studies indicate that low salinity can impact *F. grandis* condition and reproduction (Brown et al. 2012, Patterson et al. 2012).

Another main driver of variation in *F. grandis* condition identified in this study was catchment land use. Urbanization within the catchments of collection sites could introduce contaminants through runoff (Sanger et al. 1999, Holland et al. 2004, Sanger et

al. 2004, Van Dolah et al. 2008, Arismendez et al. 2009, Yang 2012) which could negatively impact aquatic ecosystem health. Also, much of the urbanization, along with some industry, is along the upper bay where low salinity sites are located. This could confound which of salinity or urbanization is reducing the l-w ratio. Wedge et al. (2015) found an effect of urbanization on *F. grandis* condition independent of salinity gradients. The data from this study combined with the findings of Wedge et al. (2015) suggests that both salinity and urbanization are important drivers of *F. grandis* condition.

The overall limited and unequal sample sizes between sites could bias the data. This could limit the ability to distinguish the variables driving the patterns by leading to confounding among variables. The number of *F. grandis* collected from low salinity sites in the upper bay were low by comparison to the number collected from the high salinity coastal fringing marshes. In addition, the low salinity sites mostly contained heavily urbanized catchments and the coastal fringing marsh sites contained mostly natural catchments. As mentioned above, this could confound salinity and urbanization.

Another limitation to this study is the combination of male and female *F. grandis* during analysis. While growth rates between genders of *F. grandis* do not differ significantly, indices such as GSI would be expected to be much different in females vs males. The lack of separation of gender could mask patterns of condition in such indices.

The main challenges that were present during this study were the variation introduced due to collecting *F. grandis* throughout the spawning season and the methodology of using bomb calorimetry to determine caloric content of *F. grandis*. There are two methods that can reduce variability caused by collecting *F. grandis* during spawning season. The first is to time collections so that they fall at the midpoint of *F.*

grandis spawning cycle. *F. grandis* spawning peaks around the full or new moon phase (Hsiao and Meier 1989, Green 2013). Therefore, sampling during the neap tide phase would minimize the chance of collecting *F. grandis* that have just spawned mixed in with those that are just about to spawn. The other method would be to significantly increase sample size. This would allow for a subset of *F. grandis* identified to be at the same point in the spawning cycle to be analyzed. Categorization of an individual's reproductive stage could be done through macroscopic examination of the gonads (Ferreri et al. 2009).

While analyzing a subset of *F. grandis* at the same stage in the spawning cycle would reduce the variability in body condition due to spawning, catch per unit effort was low in the low salinity, upper bay sites and thus increasing collection size could be difficult. One potential cause of the low CPUE was a 40-year flood event just before Spring sampling began (Scheurich 2020). A solution for this is to sample during a drier year without floods. With less freshwater input from the rivers that feed into Mobile Bay, the upper bay will be more habitable for *F. grandis*, which should increase CPUE. Another potential solution is to sample sites further east or west in a similar salinity regime but with clear impacts, such as industrial areas around Pascagoula, and more urbanized high salinity sites in Florida. This could help to separate the effects of salinity, urbanization, and industrial inputs.

There are two recommendations for improving the use of bomb calorimetry in future studies. First, ensure that the sample is homogenized to the appropriate level so that replicate sub-samples produce adequately precise results, and test the repeatability of the procedure on a few samples before running all the samples. This will ensure that the instrument is producing reliable and repeatable results. Second, ensure the sample is

completely dried after homogenization. Any moisture absorbed by the homogenized sample during the homogenization process will skew the results of the bomb calorimeter since the water will add to the sample weight but not to its caloric content. These recommendations should improve the accuracy and reliability of the results obtained from bomb calorimetry.

Future studies should focus more sampling effort at high impact sites. This could be accomplished by attempting more collections around IPC and Downtown Mobile sites or by extending collections further along the coast to include areas such as Pascagoula. While this study identified urbanization within site catchment as a driver of variation in *F. grandis* condition, few targeted, potentially impacted sites were sampled successfully. An increase in data in from these sites could alter or further support the trends identified by the CART analyses.

Another way in which future studies could utilize *F. grandis* is to develop a BACI framework. Utilizing BACI requires that the affected site be sampled before a major event (Underwood 1991, Smith 2002, Sheaves et al. 2012). Regular monitoring of *F. grandis* condition could provide the framework needed to implement a BACI study in the event of an environmental threat, such as an oil spill or a hurricane. Utilizing a BACI design would account for any background changes not related to the environmental threat, such as salinity or existing urbanization effects seen in this study.

Detecting the effects of dredging the ship channel and disposal of the material is another area in which *F. grandis* could be utilized. A recent US Army Corps of Engineers paper evaluated potential impacts of expanding the shipping channel on the aquatic resources of Mobile Bay (Berkowitz et al. 2020). Berkowitz et al. (2020) concluded that

since the area of impact is already adjusted to shifts in salinity and other factors, that minimal impact would be seen from such an expansion. This conclusion can be supported by conducting a BACI study on *F. grandis* condition metrics from nearby salt marshes that would be within the area of impact.

Another major use for *F. grandis* could be monitoring the success of coastal restoration projects such as living shorelines. Living shorelines aim to reduce erosion, protect or restore natural shoreline habitat, and maintain coastal processes through the use of natural vegetation with some supporting structures rather than using shoreline hardening techniques (Dutta et al. 2021). A large amount of money has been invested from government agencies and landowners to implement living shorelines (Gittman and Scyphers 2017) and methods to determine the general health of these restored habitats are needed. Killifish condition metrics could be a cost-effective indicator of the general health of these restored ecosystems relative to multiple control sites.

Overall, *F. grandis* shows promise as an environmental indicator species. *F. grandis* shows high site fidelity (Nelson et al. 2014), a measurable response to varying environmental conditions (this study; Wedge et al. 2015), and is an ecologically important species (Rozas and Reed 1993), all of which are needed to be a good indicator species. This study showed that some variation in *F. grandis* body condition can be explained by environmental factors, such as salinity and catchment land use. Since *F. grandis* shows potential as an environmental indicator species, it could be used in future studies as a relatively inexpensive indicator of environmental health.

REFERENCES

REFERENCES

Alexander, R. B., Smith, R. A., Schwartz, G. E., Preston, S. D., Brakebill, J. W., Srinivasan, R., & Pacheco, P. A. (2001). Atmospheric Nitrogen Flux From the Watersheds of Major Estuaries of the United States: An Application of the SPARROW Watershed Model. In Valigura, R. A., Alexander, R. B., Castro, M. S., Meyers, T. P., Paerl, H. W., Stacey, P. E., Turner, R. E. (Eds.), *Nitrogen Loading in Coastal Water Bodies: An Atmospheric Prospective* (pp 119-170). American Geophysical Union.

Anderson, & R., Gutreuter, S., (1983). Length, Weight and Associated Structural Indices. In: Fisheries Techniques. Nielsen, L., and Johnson, D. (Eds). American Fisheries Society, Bethesda, MD, 283–300.

Ando, S., Mori, Y., Nakamura, K., Sugawara, R. (1993). Characteristics of Lipid Accumulation Types in Five Species of Fish. *Nippon Suisan Gakkaishi*, *59*(9), 1559-1564.

Arismendez, S. S., Kim, H. C., Brenner, J., & Montagna, P. A. (2009). Application of Watershed Analyses and Ecosystem Modeling to Investigate Land–Water Nutrient Coupling Processes in the Guadalupe Estuary, Texas. *Ecological Informatics*, *4*(4), 243-253.

Arrington, D. A., Davidson, B. K., Winemiller, K. O., & Layman, C. A. (2006). Influence of Life History and Seasonal Hydrology on Lipid Storage in Three Neotropical Fish Species. *Journal of Fish Biology*, *68*(5), 1347-1361.

Baker, R., Fry, B., Rozas, L. P., & Minello, T. J. (2013). Hydrodynamic regulation of salt marsh contributions to aquatic food webs. *Marine Ecology Progress Series, 490*, 37-52.

Barber, B. J., & Blake, N. J. (1981). Energy Storage and Utilization in Relation to Gametogenesis in *Argopecten irradians concentricus* (Say). *Journal of Experimental Marine Biology and Ecology*, *52*(2-3), 121-134.

Barton, B. A., Morgan, J. D., & Vijayan, M. M. (2002). Physiological and Condition-Related Indicators of Environmental Stress in Fish. In Adams, S. M. (Ed.), *Biological Indicators of Aquatic Ecosystem Stress* (pp 111-148). Bethesda, USA: American Fisheries Society.

Baya, E. E., Yokel, L. S., Pinyerd III, C. A., Blancher, E. C., Sklenar, S. A., & Isphording, W. C. (1998). Preliminary Characterization of Water Quality of the Mobile Bay National Estuary (MBNEP) Study Area: Mobile. *Ala., Mobile Bay National Estuary Program*.

Berkowitz, J. F., Altman, S., Reine, K. J., Wilbur, D., Kjelland, M. E., Gerald, T. K., ... & Trahan, C. J. (2020). Evaluation of the potential impacts of the proposed Mobile Harbor navigation channel expansion on the aquatic resources of Mobile Bay, Alabama.

Bernard, M. B., Cole, P., Kobelt, A., Horne, P. A., Altmann, J., Wratten, S. D., & Yen, A. L. (2010). Reducing the Impact of Pesticides on Biological Control in Australian Vineyards: Pesticide Mortality and Fecundity Effects on an Indicator Species, the Predatory Mite *Euseius victoriensis* (Acari: Phytoseiidae). *Journal of Economic Entomology*, *103*(6), 2061-2071.

Brewer, S. K., Rabeni, C. F., & Papoulias, D. M. (2008). Comparing Histology and Gonadosomatic Index for Determining Spawning Condition of Small□Bodied Riverine Fishes. *Ecology of Freshwater Fish*, *17*(1), 54-58.

Brown, C. A., Galvez, F., & Green, C. C. (2012). Embryonic Development and Metabolic Costs in Gulf killifish *Fundulus grandis* Exposed to Varying Environmental Salinities. *Fish Physiology and Biochemistry*, *38*(4), 1071-1082.

Burnett, K. G., Bain, L. J., Baldwin, W. S., Callard, G. V., Cohen, S., Di Giulio, R. T., ... & Crawford, D. L. (2007). *Fundulus* as the Premier Teleost Model in Environmental Biology: Opportunities for New Insights Using Genomics. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, *2*(4), 257-286.

Chambers, J. (1992). Coastal Degradation and Fish Population Losses. In R. Stroud (Ed.), *Stemming the Tide of Coastal Fish Habitat Loss* (pp. 45-52). Savannah, GA: National Coalition for Marine Conservation.

Coogan, J., & Dzwonkowski, B. (2018). Observations of Wind Forcing Effects on Estuary Length and Salinity Flux in a River-Dominated, Microtidal Estuary, Mobile Bay, Alabama. *Journal of Physical Oceanography*, *48*(8), 1787-1802.

De'ath, G., & Fabricius, K. E. (2000). Classification and regression trees: a powerful yet simple technique for ecological data analysis. *Ecology, 81*(11), 3178-3192.

Dean, T. L., & Richardson, J. (1999). Responses of Seven Species of Native Freshwater Fish and a Shrimp to Low Levels of Dissolved Oxygen. *New Zealand Journal of Marine and Freshwater Research*, *33*(1), 99-106.

Dorato, M. A., & Engelhardt, J. A. (2005). The No-Observed-Adverse-Effect-Level in Drug Safety Evaluations: Use, Issues, and Definition (s). *Regulatory Toxicology and Pharmacology*, *42*(3), 265-274.

Dubansky, B., Whitehead, A., Miller, J. T., Rice, C. D., & Galvez, F. (2013). Multitissue Molecular, Genomic, and Developmental Effects of the Deepwater Horizon Oil Spill on Resident Gulf killifish (*Fundulus grandis*). *Environmental Science & Technology*, *47*(10), 5074-5082.

Dutta, S., Biber, P. D., & Boyd, C. A. (2021). Nearshore Sediment Comparisons among Natural, Living, and Armored Shorelines in Mobile Bay, Alabama. *Southeastern Naturalist*, *20*(1), 135-151.

Elliott, M., Whitfield, A. K., Potter, I. C., Blaber, S. J., Cyrus, D. P., Nordlie, F. G., & Harrison, T. D. (2007). The guild approach to categorizing estuarine fish assemblages: a global review. *Fish and fisheries, 8*(3), 241-268.

Ellis, W. L., & Bell, S. S. (2013). Intertidal Fish Communities May Make Poor Indicators of Environmental Quality: Lessons from a Study of Mangrove Habitat Modification. *Ecological Indicators*, *24*, 421-430.

Environmental Health Center. (1998). *Coastal Challenges: A Guide to Coastal and Marine Issues.* Washington, DC: A Division of the National Safety Council.

Ferreri, R., Basilone, G., D'Elia, M., Traina, A., Saborido□Rey, F., & Mazzola, S. (2009). Validation of Macroscopic Maturity Stages According to Microscopic Histological Examination for European Anchovy. *Marine Ecology*, *30*, 181-187.

Fournet, M. E., Stabenau, E., & Rice, A. N. (2019). Relationship Between Salinity and Sonic Fish Advertisement Behavior in a Managed Sub-Tropical Estuary: Making the Case for an Acoustic Indicator Species. *Ecological Indicators*, *106*, 105531.

Gérard, C., Blanc, A., & Costil, K. (2003). Potamopyrgus antipodarum (Mollusca: Hydrobiidae) in continental aquatic gastropod communities: impact of salinity and trematode parasitism. *Hydrobiologia, 493*(1), 167-172.

Gittman, R. K., & Scyphers, S. B. (2017). The Cost of Coastal Protection: A Comparison of Shore Stabilization Approaches. *Shore and Beach*, *85*, 19-24.

Green, C. (2013). Intensive (Non-Pond) Culture of Gulf Killifish. *SRAC Publication*, *1202*.

Gupta, S. K., & Singh, J. (2011). Evaluation of mollusc as sensitive indicator of heavy metal pollution in aquatic system: a review. *IIOAB J, 2*(1), 49-57.

Handeland, S. O., Imsland, A. K., & Stefansson, S. O. (2008). The Effect of Temperature and Fish Size on Growth, Feed Intake, Food Conversion Efficiency and Stomach Evacuation Rate of Atlantic Salmon Post-Smolts. *Aquaculture*, *283*(1-4), 36-42.

Harrington, R. W., & Harrington, E. S. (1961). Food selection among fishes invading a high subtropical salt marsh: from onset of flooding through the progress of a mosquito brood. *Ecology*, *42*, 646-666.

Holland, A. F., Sanger, D. M., Gawle, C. P., Lerberg, S. B., Santiago, M. S., Riekerk, G. H., ... & Scott, G. I. (2004). Linkages Between Tidal Creek Ecosystems and the Landscape and Demographic Attributes of their Watersheds. *Journal of Experimental Marine Biology and Ecology*, *298*(2), 151-178.

Hsiao, S. M., & Meier, A. H. (1989). Comparison of semilunar cycles of spawning activity in Fundulus grandis and F. heteroclitus held under constant laboratory conditions. *Journal of Experimental Zoology, 252*(3), 213-218.

Jaume, D., & Boxshall, G. A. (2007). Global diversity of cumaceans & tanaidaceans (Crustacea: Cumacea & Tanaidacea) in freshwater. In *Freshwater Animal Diversity Assessment* (pp. 225-230). Springer, Dordrecht.

Jensen, O. P., Martin, C. W., Oken, K. L., Fodrie, F. J., López-Duarte, P. C., Able, K. W., & Roberts, B. J. (2019). Simultaneous estimation of dispersal and survival of the gulf killifish *Fundulus grandis* from a batch-tagging experiment. *Marine Ecology Progress Series*, 624, 183-194.

Kneib, R. T., & Stiven, A. E. (1978). Growth, Reproduction, and Feeding of *Fundulus heteroclitu*s (L.) on a North Carolina Salt Marsh. *Journal of Experimental Marine Biology and Ecology*, *31*(2), 121-140.

Landry, C. A., Steele, S. L., Manning, S., & Cheek, A. O. (2007). Long Term Hypoxia Suppresses Reproductive Capacity in the Estuarine Fish, *Fundulus grandis*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *148*(2), 317-323.

Laurén, D. J., & Wails, D. (2018). Liver Structural Alterations Accompanying Chronic Toxicity in Fishes: Potential Biomarkers of Exposure. In *Biomarkers of Environmental Contamination* (pp. 17-57). Crc Press.

Lindenmayer, D. B., & Likens, G. E. (2011). Direct Measurement Versus Surrogate Indicator Species for Evaluating Environmental Change and Biodiversity Loss. *Ecosystems*, *14*(1), 47-59.

Loh, W. Y. (2014). Fifty years of classification and regression trees. *International Statistical Review*, *82*(3), 329-348.

Martínez, M. L., Landry, C., Boehm, R., Manning, S., Cheek, A. O., & Rees, B. B. (2006). Effects of Long-Term Hypoxia on Enzymes of Carbohydrate Metabolism in the Gulf Killifish, *Fundulus grandis*. *Journal of Experimental Biology*, *209*(19), 3851-3861.

McAdam, D. S. O., Liley, N. R., & Tan, E. S. (1999). Comparison of Reproductive Indicators and Analysis of the Reproductive Seasonality of the Tinfoil Barb, *Puntius schwanenfeldii*, in the Perak River, Malaysia. *Environmental Biology of Fishes*, *55*(4), 369-380.

McBride, R. S., Somarakis, S., Fitzhugh, G. R., Albert, A., Yaragina, N. A., Wuenschel, M. J., ... & Basilone, G. (2015). Energy Acquisition and Allocation to Egg Production in Relation to Fish Reproductive Strategies. *Fish and Fisheries*, *16*(1), 23-57.

McLusky, D. S., & Elliott, M. (2004). *The estuarine ecosystem: ecology, threats and management*. OUP Oxford.

Mills, L. S., Soulé, M. E., & Doak, D. F. (1993). The Keystone-Species Concept in Ecology and Conservation. *BioScience*, *43*(4), 219-224.

Moles, A., & Rice, S. D. (1983). Effects of Crude Oil and Naphthalene on Growth, Caloric Content, and Fat Content of Pink Salmon Juveniles in Seawater. *Transactions of the American Fisheries Society*, *112*(2A), 205-211.

Nelson, T. R., Sutton, D., & DeVries, D. R. (2014). Summer Movements of the Gulf Killifish (*Fundulus grandis*) in a Northern Gulf of Mexico Salt Marsh. *Estuaries and Coasts*, *37*(5), 1295-1300.

Nunes, C., Silva, A., Marques, V., & Ganias, K. (2011). Integrating Fish Size, Condition, and Population Demography in the Estimation of Atlantic Sardine Annual Fecundity. *Ciencias Marinas*, *37*(4B), 565-584.

Odum, W. E., & Heald, E. J. (1972). Trophic analyses of an estuarine mangrove community. *Bulletin of Marine Science, 22*(3), 671-738.

Paine, R. T. (1969). A Note on Trophic Complexity and Community Stability. *The American Naturalist*, *103*(929), 91-93.

Park, K., Kim, C. K., & Schroeder, W. W. (2007). Temporal Variability in Summertime Bottom Hypoxia in Shallow Areas of Mobile Bay, Alabama. *Estuaries and Coasts*, *30*(1), 54-65.

Parr Instrument Company. (2008). *Oxygen Bomb Calorimeter 1341: Operating Instruction Manual.* Moline, Illinois: Parr Instrument Company.

Patterson, J., Bodinier, C., & Green, C. (2012). Effects of Low Salinity Media on Growth, Condition, and Gill Ion Transporter Expression in Juvenile Gulf killifish, *Fundulus grandis*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *161*(4), 415-421.

Plaza, G., Sakaji, H., Honda, H., Hirota, Y., & Nashida, K. (2007). Spawning Pattern and Type of Fecundity in Relation to Ovarian Allometry in the Round Herring *Etrumeus teres*. *Marine Biology*, *152*(5), 1051-1064.

Qiu, J. W., & Qian, P. Y. (1998). Combined effects of salinity and temperature on juvenile survival, growth and maturation in the polychaete Hydroides elegans. *Marine Ecology Progress Series, 168*, 127-134.

Rice, J. (2003). Environmental health indicators. *Ocean & Coastal Management*, *46*(3-4), 235-259.

Rinchard, J., & Kestemont, P. (1996). Comparative Study of Reproductive Biology in Single□and Multiple□Spawner Cyprinid Fish. I. Morphological and histological features. *Journal of Fish Biology*, *49*(5), 883-894.

Roff, D. A. (1983). An allocation model of growth and reproduction in fish. *Canadian Journal of Fisheries and Aquatic Sciences*, *40*(9), 1395-1404.

Rozas, L. P., & LaSalle, M. W. (1990). A Comparison of the Diets of Gulf killifish, *Fundulus grandis* Baird and Girard, Entering and Leaving a Mississippi Brackish Marsh. *Estuaries*, *13*(3), 332-336.

Rozas, L. P., & Minello, T. J. (2011). Variation in Penaeid Shrimp Growth Rates Along an Estuarine Salinity Gradient: Implications for Managing River Diversions. *Journal of Experimental Marine Biology and Ecology*, *397*(2), 196-207.

Rozas, L. P., & Reed, D. J. (1993). Nekton Use of Marsh-Surface Habitats in Louisiana (USA) Deltaic Salt Marshes Undergoing Submergence. *Marine Ecology-Progress Series*, *96*, 147-147.

Sanger, D. M., Holland, A. F., & Scott, G. I. (1999). Tidal Creek and Salt Marsh Sediments in South Carolina Coastal Estuaries: II. Distribution of Organic

Contaminants. *Archives of Environmental Contamination and Toxicology*, *37*(4), 458-471.

Sanger, D. M., Holland, A. F., & Hernandez, D. L. (2004). Evaluation of the Impacts of Dock Structures and Land Use on Tidal Creek Ecosystems in South Carolina Estuarine Environments. *Environmental Management*, *33*(3), 385-400.

Scheurich, H. (2020, February 26). *Flood water on Tensaw River reaches highest levels since 1980*. Fox 10 News. https://www.fox10tv.com/news/baldwin_county/flood-water-on-tensaw-river-reaches-highest-levels-since-1980/article_6dd569ea-58e7-11ea-8f6f-6b7f1ce47aeb.html

Sheaves, M., Johnston, R., & Connolly, R. M. (2012). Fish Assemblages as Indicators of Estuary Ecosystem Health. *Wetlands Ecology and Management*, *20*(6), 477-490.

Smith, E. P. (2002). BACI Design. *Encyclopedia of environmetrics*, *1*, 141-148.

Sogard, S. M. (1997). Size-Selective Mortality in the Juvenile Stage of Teleost Fishes: A Review. *Bulletin of Marine Science*, *60*(3), 1129-1157.

Stewart, J. (2020). *Watershed-Estuary Dynamics in the Mobile River Watershed-Mobile Bay Estuary (mr-mb) Continuum Examined by Combined Geochemical and Satellite Approaches*. The University of Alabama.

Tsoi, K. H., Chiu, K. M., & Chu, K. H. (2005). Effects of temperature and salinity on survival and growth of the amphipod Hyale crassicornis (Gammaridea, Hyalidae). *Journal of Natural History, 39*(4), 325-336.

Underwood, A. J. (1991). Beyond BACI: experimental designs for detecting human environmental impacts on temporal variations in natural populations. *Marine and Freshwater Research*, *42*(5), 569-587.

Van Dolah, R. F. (1978). Factors Regulating the Distribution and Population Dynamics of the Amphipod *Gammarus palustris* in an Intertidal Salt Marsh Community. *Ecological Monographs*, *48*(2), 191-217.

Van Dolah, R. F., Riekerk, G. H., Bergquist, D. C., Felber, J., Chestnut, D. E., & Holland, A. F. (2008). Estuarine Habitat Quality Reflects Urbanization at Large Spatial Scales in South Carolina's Coastal Zone. *Science of the Total Environment*, *390*(1), 142-154.

Vastano, A. R., Able, K. W., Jensen, O. P., López-Duarte, P. C., Martin, C. W., & Roberts, B. J. (2017). Age Validation and Seasonal Growth Patterns of a Subtropical Marsh Fish: The Gulf Killifish, *Fundulus grandis*. *Environmental Biology of Fishes*, *100*(10), 1315-1327.

Vondracek, B., Giese, B. D., & Henry, M. G. (1996). Energy Density of Three Fishes from Minnesota Waters of Lake Superior. *Journal of Great Lakes Research*, *22*(3), 757-764.

Wagenmakers, E. J., & Farrell, S. (2004). AIC model selection using Akaike weights. *Psychonomic bulletin & review*, *11*(1), 192-196.

Wedge, M., Anderson, C. J., & DeVries, D. (2015). Evaluating the Effects of Urban Land Use on the Condition of Resident Salt Marsh Fish. *Estuaries and Coasts*, *38*(6), 2355-2365.

Whitehead, A., Dubansky, B., Bodinier, C., Garcia, T. I., Miles, S., Pilley, C., ... & Galvez, F. (2012). Genomic and Physiological Footprint of the Deepwater Horizon Oil Spill on Resident Marsh Fishes. *Proceedings of the National Academy of Sciences*, *109*(50), 20298-20302.

Wolfe, B. W., & Lowe, C. G. (2015). Movement Patterns, Habitat Use and Site Fidelity of the White Croaker (*Genyonemus lineatus*) in the Palos Verdes Superfund Site, Los Angeles, California. *Marine Environmental Research*, *109*, 69-80.

Yan, J., Liao, K., Wang, T., Mai, K., Xu, W., & Ai, Q. (2015). Dietary Lipid Levels Influence Lipid Deposition in the Liver of Large Yellow Croaker (*Larimichthys crocea*) by Regulating Lipoprotein Receptors, Fatty Acid Uptake and Triacylglycerol Synthesis and Catabolism at the Transcriptional Level. *PloS one*, *10*(6), e0129937.

Yang, X. (2012). An assessment of landscape characteristics affecting estuarine nitrogen loading in an urban watershed. *Journal of environmental management*, *94*(1), 50-60.

Zu Ermgassen, P. S., Spalding, M. D., Grizzle, R. E., & Brumbaugh, R. D. (2013). Quantifying the loss of a marine ecosystem service: filtration by the eastern oyster in US estuaries. *Estuaries and coasts*, *36*(1), 36-43.

APPENDICES

Appendix A Sampling Permits and Approvals

INSTITUTIONAL ANIMAL CARE
AND USE COMMITTEE

UNIVERSITY OF
SOUTH ALABAMA

TELEPHONE: (251) 460-6863
AD 240
MOBILE, ALABAMA 36688

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

Date: June 27, 2019

Date of Review: June 20, 2019

Protocol #: [1437888-2]
Category: *D*

Protocol Title: The condition of marsh-resident killifish Fundulus grandis as indicators of ecosystem health
Responsible Investigator: Ronald Baker, PhD

Type of Protocol:

[X] RESEARCH

[ ] DEMONSTRATION

[ ] TRAINING

IACUC Approval Authorization:

The Institutional Animal Care and Use Committee, authorized under the OLAW assurance number A3288-01, reviewed this project and, on the basis of this review, the IACUC has approved this protocol as follows:

[X] Approval for three (3) years, subject to annual monitoring and review.

[ ] Approval with *Restrictions noted below:*

The Principal Investigator (PI) is responsible for this project in accordance with all federal, state and local laws and regulations, NIH/ILR guidelines, and established institutional policies and procedures.

In conducting this project, the PI agrees to have the laboratory animals available for examination by IACUC members or Department of Comparative Medicine personnel at all times during the project. Anytime any animal is deemed to be in unacceptable distress or is receiving inhumane treatment, in the professional opinion of the attending veterinarian, that animal may be subjected to immediate euthanasia and/or the procedure immediately halted. In such cases the IACUC will be informed of any action taken.

The Director of University Biological Resources may suspend a protocol for cause until the IACUC has reviewed the incidents leading to the suspension.

IACUC approval of this protocol does not guarantee availability of resources or services from the Department of Comparative Medicine.

Restrictions:

Generated on IRBNet

A1. IACUC approval form.

STATE OF ALABAMA
DEPARTMENT OF CONSERVATION AND NATURAL RESOURCES
MARINE RESOURCES DIVISION
POST OFFICE BOX 189
DAUPHIN ISLAND, ALABAMA 36528
TEL (251) 861-2882
FAX (251) 861-8741
marine.resources@dcnr.alabama.gov

Kay Ivey
GOVERNOR

Christopher M. Blankenship
COMMISSIONER

Edward F. Poolos
DEPUTY COMMISSIONER

Our mission is to manage the State's marine fishery resources through research, enforcement, and education for the maximum benefit of the resources and the citizens of Alabama.

M. Scott Bannon
Director
MARINE RESOURCES DIVISION

October 23, 2019

Dr. Ronald Baker, Ph.D.
Assistant Professor
University of South Alabama
Dauphin Island Sea Lab
101 Bienville Boulevard
Dauphin Island, AL 36528

Dear Dr. Baker:

This letter will serve as your permit within Alabama's bays and territorial seas for the collection of fish and crustaceans. The following equipment may be used for collection: minnow traps, cast nets, seine nets, 16' otter trawl, and hook and line. This permit is valid from **November 1, 2019 through October 31, 2020.**

The DISL staff members working with the Baker Lab which includes individuals, post-docs, students, research technicians, and interns will be allowed to collect the fish and crustaceans.

This letter must be kept on file at your facility during the permit period and a copy of this letter must be available for inspection at any site and/or in the possession of the captain or operator of any vessel or vehicle participating in this activity.

We ask that you notify our office at 251-861-2882 prior to operations to enable us to answer any questions the public may have about your operations and keep us abreast of what you are doing.

In the event of interactions with federally protected marine species while conducting sampling under this permit, the principal investigator or designated representative of your institution is required to notify the appropriate federal agency and ADCNR/MRD within 24 hours of the interaction. This requirement includes lethal and non-lethal interactions. Species include, but are not limited to; dolphins, manatees, sea turtles, sturgeon and sea birds. It is the responsibility of the permit holder to ensure all preventative measures are taken to avoid interactions.

I hope this helps you with your plans. If you have any questions, please contact me or Marine Resources Division Office at 251-861-2882.

Sincerely

Colonel M. Scott Bannon, Director
Marine Resources Division

The Department of Conservation and Natural Resources does not discriminate on the basis of race, color, religion, age, sex, national origin, disability, pregnancy, genetic information or veteran status in its hiring or employment practices nor in admission to, access to, or operations of its programs, services, or activities.
www.outdooralabama.com

A2. DCNR collection permit

Appendix B Local Habitat and Watershed Maps

Figure B1. Airport Marsh local aquatic habitat (100m) and watershed (1km)

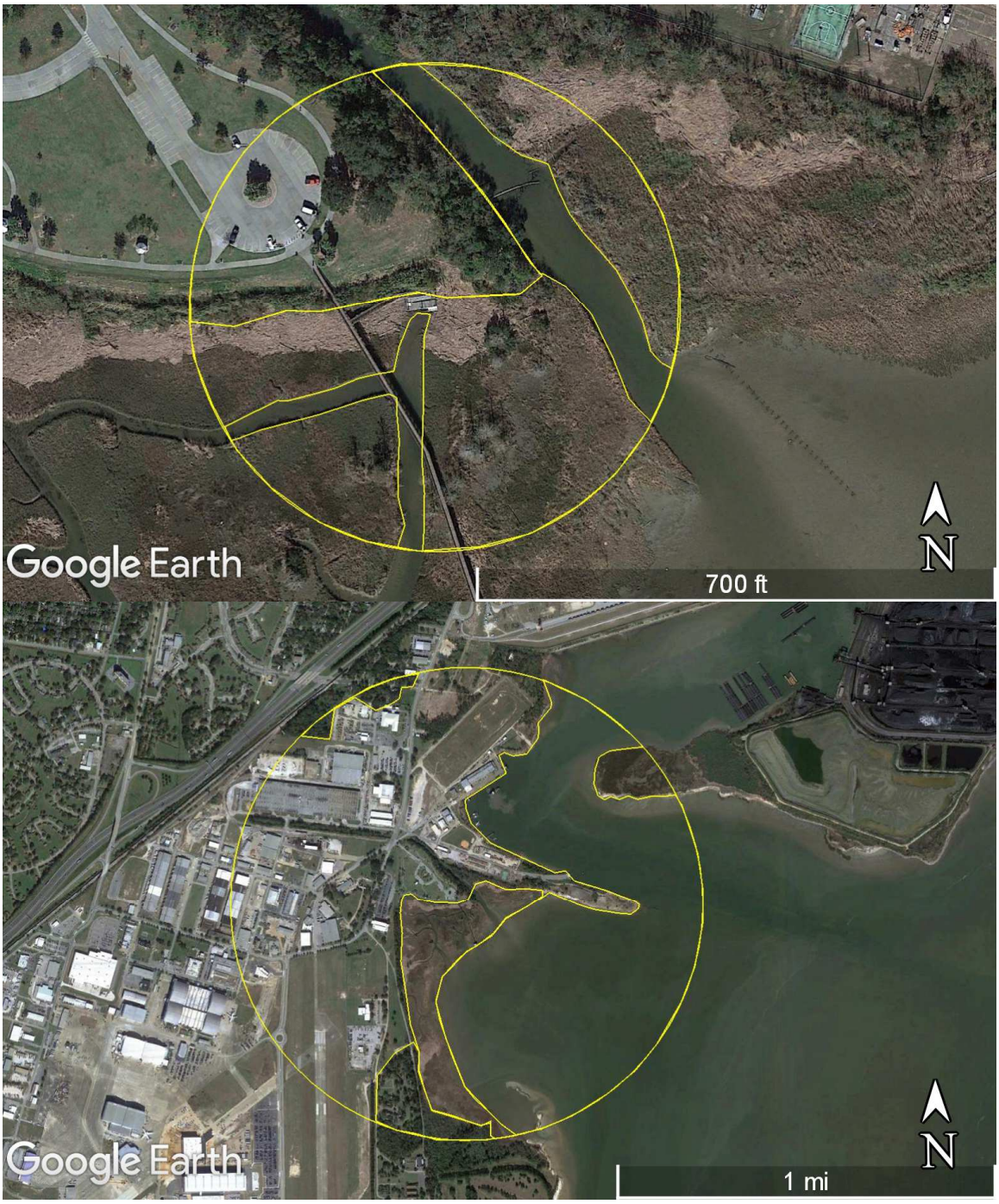

Figure B2. Arlington Park local aquatic habitat (100m) and watershed (1km)

Figure B3. Car Ferry Marsh local aquatic habitat (100m) and watershed (1km)

Figure B4. Cedar Point local aquatic habitat (100m) and watershed (1km)

Figure B5. Fort Morgan local aquatic habitat (100m) and watershed (1km)

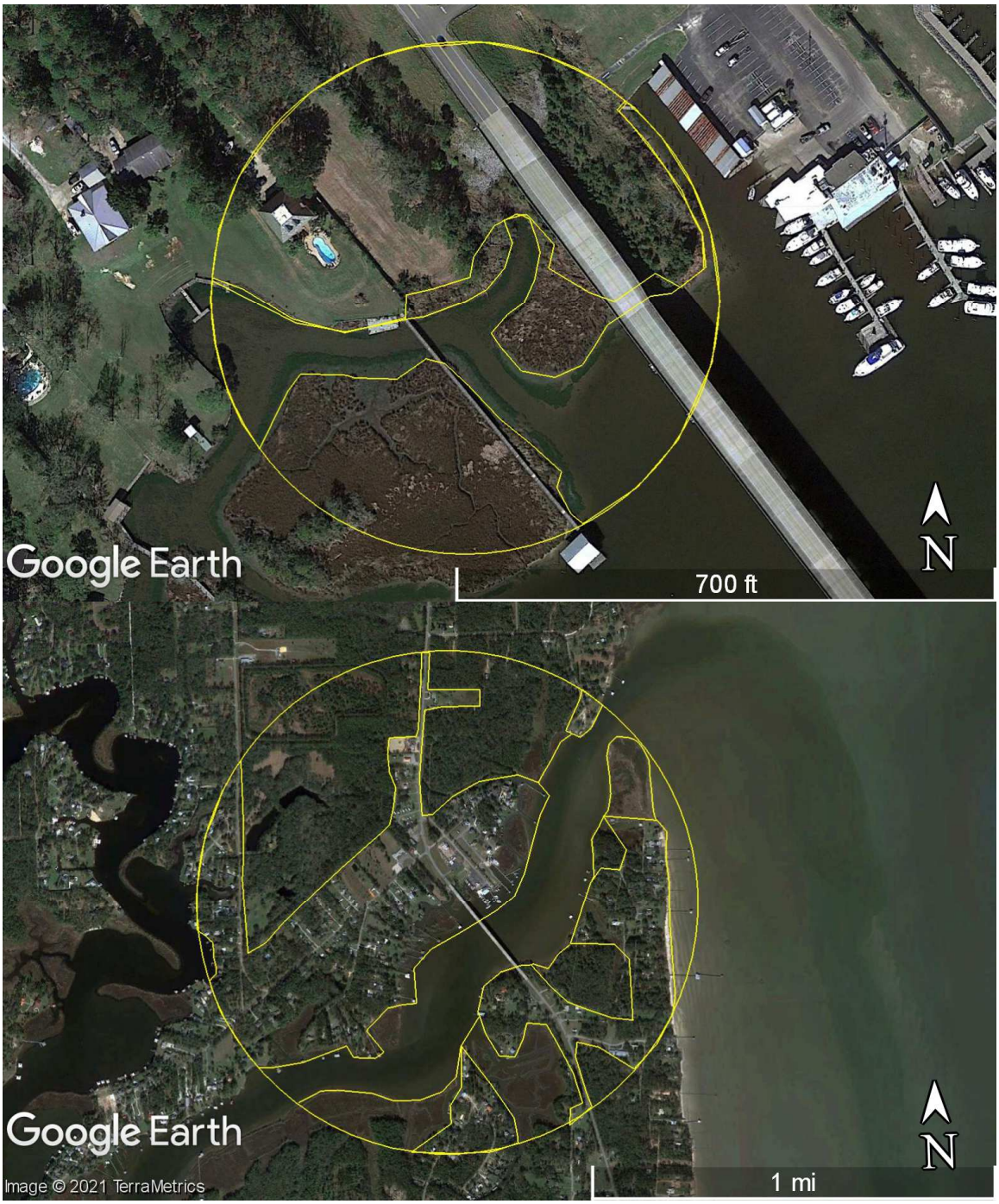

Figure B6. Fowl River local aquatic habitat (100m) and watershed (1km)

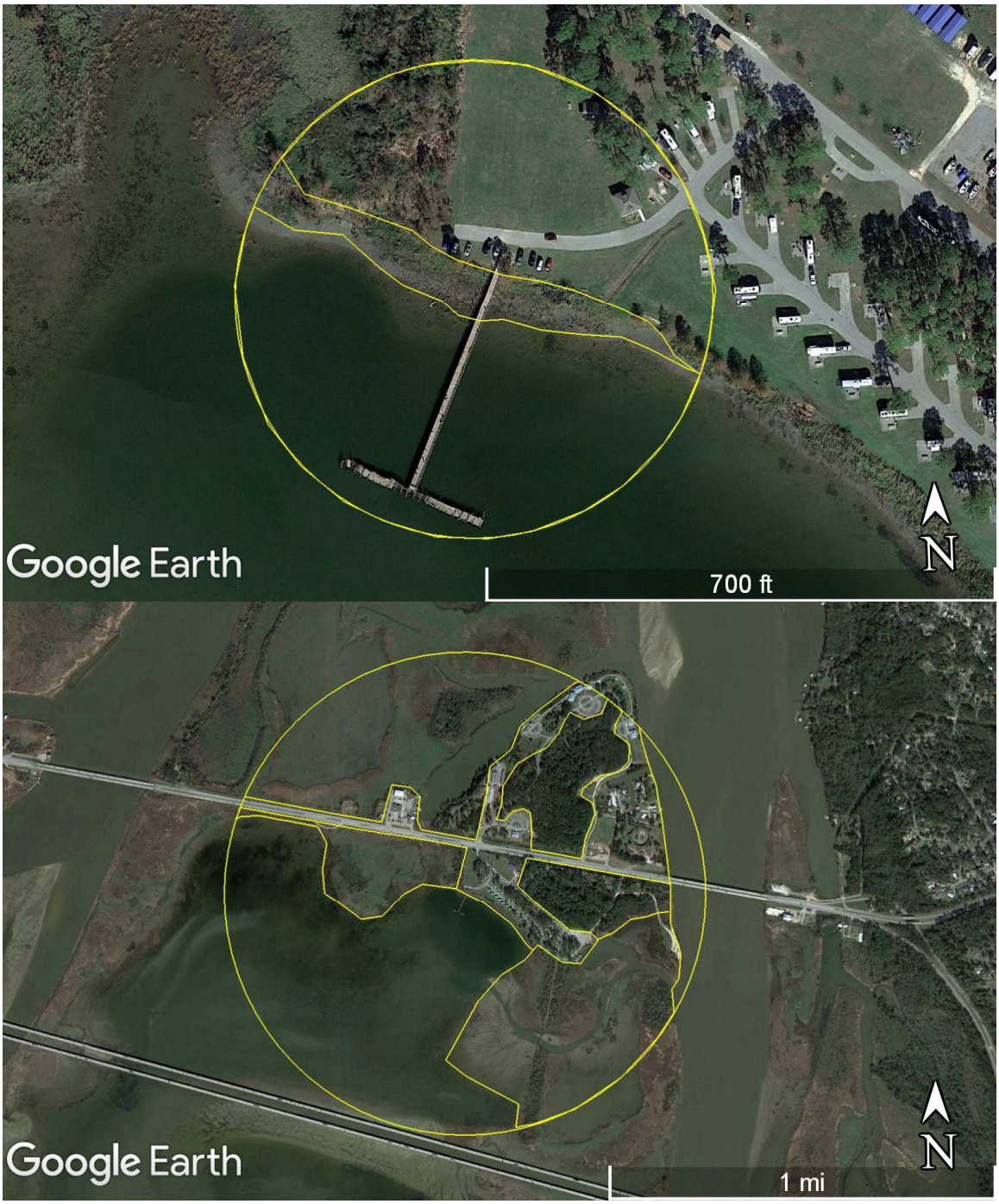

Figure B7. Meaher Park local aquatic habitat (100m) and watershed (1km)

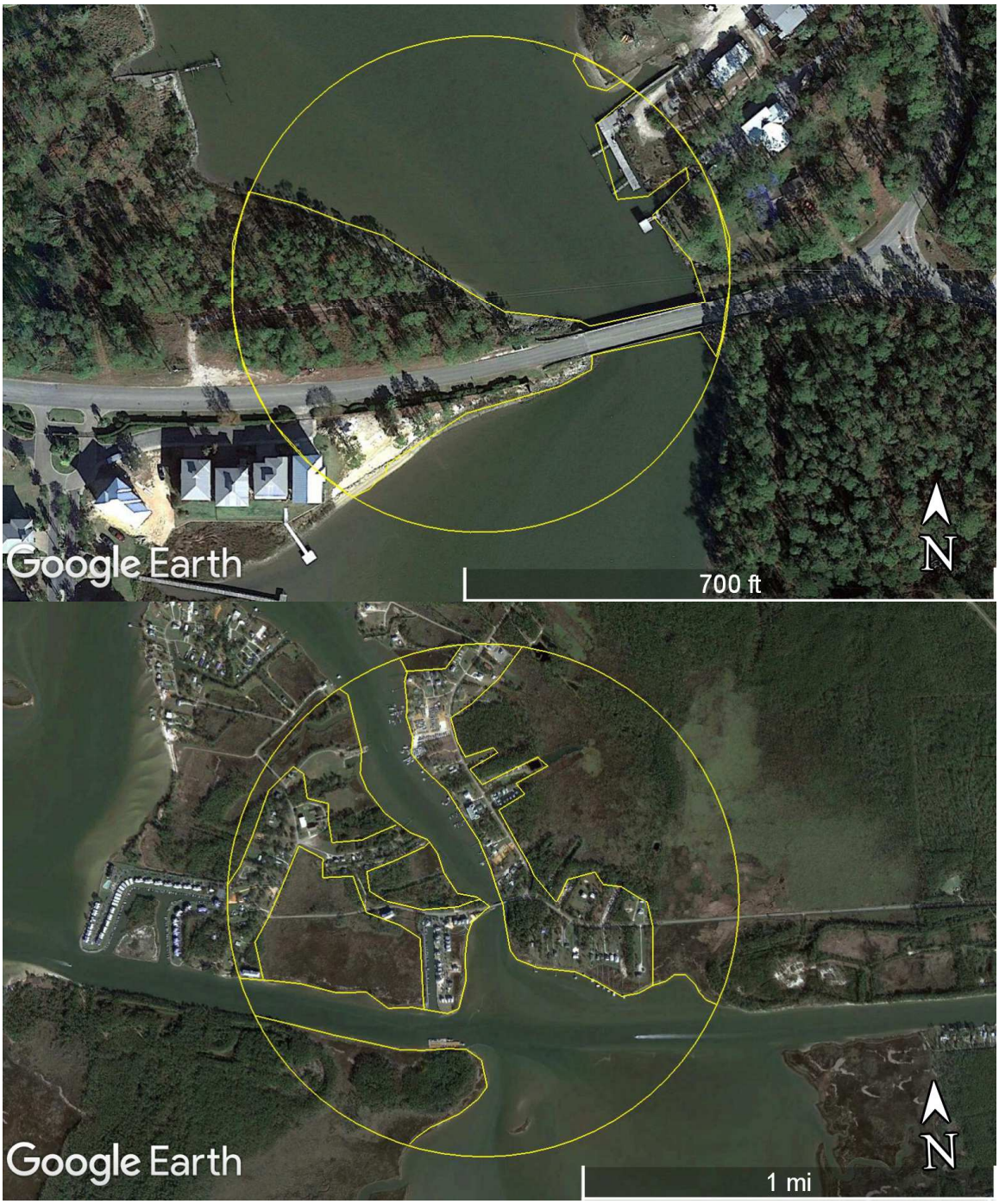

Figure B8. Oyster Bay local aquatic habitat (100m) and watershed (1km)

Figure B9. Point Clear local aquatic habitat (100m) and watershed (1km)

Figure B10. Wade Ward Park local aquatic habitat (100m) and watershed (1km)

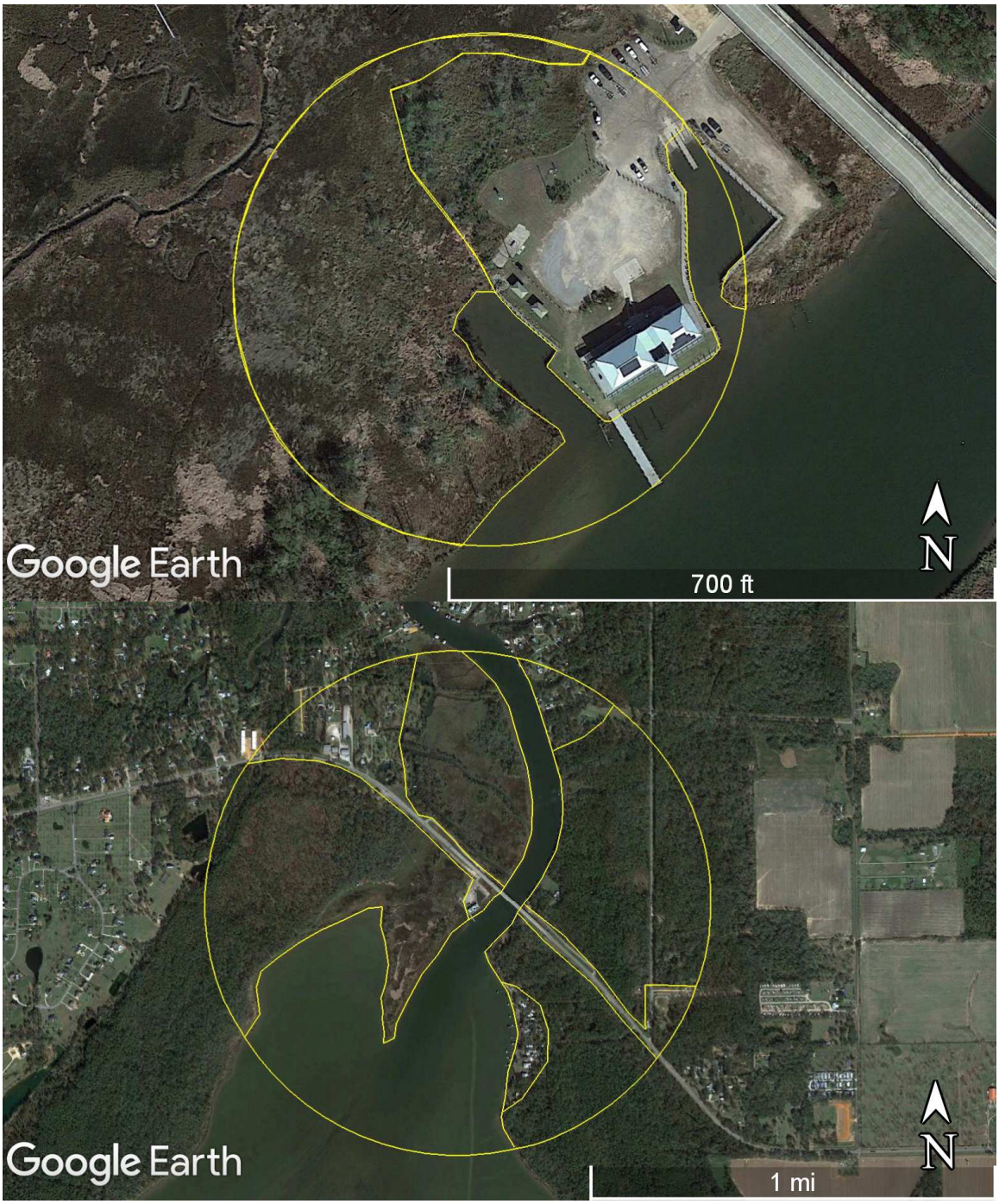

Figure B11. Weeks Bay (North) local aquatic habitat (100m) and watershed (1km)

Figure B12. Weeks Bay (South) local aquatic habitat (100m) and watershed (1km)

Figure B13. West End Beach local aquatic habitat (100m) and watershed (1km)

Figure B14. Wolf Bay local aquatic habitat (100m) and watershed (1km)

Table B15. Local habitat metrics for *Fundulus grandis* at each collection site. Total land is a percentage of total area. Open water, marsh, and tide pools are percentages of total water.

	Local Habitat Metrics (100m)			
Location	% Land	% Open Water	% Marsh	% Tide Pool
Airport Marsh	18.29	17.86	82.14	0.00
Arlington Park	29.82	0.00	100.00	0.00
Car Ferry Marsh	2.14	45.86	54.14	0.00
Cedar Point	25.36	33.11	66.89	0.00
Fort Morgan	46.98	62.18	37.82	0.00
Fowl River	45.04	49.41	50.59	0.00
Meaher Park	39.41	82.61	17.39	0.00
Oyster Bay	39.75	100.00	0.00	0.00
Point Clear	63.47	31.10	68.90	0.00
Wade Ward Park	51.48	0.00	100.00	0.00
Weeks Bay North	35.61	28.90	71.10	0.00
Weeks Bay South	45.50	75.25	24.75	0.00
West End Beach	65.04	92.70	0.00	7.30
Wolf Bay	63.61	96.31	3.69	0.00

Table B16. Catchment land use metrics for each collection site. Light urban, heavy urban, industrial, sandy beach, and forest are percentages of total land. Open water and marsh are percentages of total water. Tide pools were grouped together with marsh for these percentages.

	Catchment Land Use Metrics (1km)						
Location	% Light Urban	% Heavy Urban	% Industrial	% Open Water	% Sandy Beach	% Marsh	% Forest
Airport Marsh	93.23	0.00	0.00	82.45	6.77	17.55	0.00
Arlington Park	0.00	100.00	0.00	82.79	0.00	17.21	0.00
Car Ferry Marsh	56.70	0.00	0.00	94.24	8.97	5.76	34.33
Cedar Point	100.00	0.00	0.00	79.10	0.00	20.90	0.00
Fort Morgan	100.00	0.00	0.00	71.31	0.00	28.69	0.00
Fowl River	61.94	0.00	0.00	62.72	0.00	37.28	38.06
Meaher Park	62.59	0.00	0.00	42.13	0.00	57.87	37.41
Oyster Bay	86.64	0.00	0.00	46.10	0.00	53.90	13.36
Point Clear	100.00	0.00	0.00	98.07	0.00	1.93	0.00
Wade Ward Park	6.73	84.30	0.00	39.37	8.97	60.63	0.00
Weeks Bay North	16.88	0.00	0.00	65.65	0.00	34.35	83.12
Weeks Bay South	43.85	0.00	0.00	85.42	0.00	14.58	56.15
West End Beach	60.82	0.00	0.00	100.00	39.18	0.00	0.00
Wolf Bay	0.00	67.69	0.00	82.46	0.00	17.54	32.31

Table B17. Summer water quality metrics gathered from weather stations for each collection site. Salinity (psu), Dissolved Oxygen (DO) (mg/L), and Temperature (Temp) (°C) are averaged over the timeframe listed. Minimum Dissolved Oxygen (Min DO) (mg/L) is the lowest 5th percentile over the timeframe listed. ND represents no data.

Water Quality Metric	Timeframe	Airport Marsh	Car Ferry Marsh	Cedar Point	Fort Morgan	Fowl River
Salinity	2 weeks	19.69	16.16	16.99	18.84	17.83
Salinity	1 month	17.55	14.02	14.83	19.15	15.63
Salinity	2 months	13.49	11.54	10.40	16.20	11.08
Salinity	3 months	11.82	11.58	8.54	13.43	8.87
DO	2 weeks	90.08	90.56	82.26	96.85	87.51
DO	1 month	88.84	87.45	81.75	93.32	85.06
DO	2 months	88.82	89.22	86.11	89.77	86.98
DO	3 months	91.30	92.08	89.62	90.87	89.71
Min DO	2 weeks	4.51	4.54	3.83	4.60	4.06
Min DO	1 month	4.46	4.39	3.64	4.90	4.02
Min Do	2 months	4.44	4.54	3.95	4.65	4.07
Min DO	3 months	4.63	4.78	4.06	4.38	4.06
Temp	2 weeks	29.61	29.70	18.33	90.87	29.60
Temp	1 month	29.45	29.15	22.71	29.60	22.89
Temp	2 months	29.06	28.35	26.00	29.42	26.18
Temp	3 months	27.19	26.05	25.07	28.47	25.34

Water Quality Metric	Timeframe	Meaher Park	Oyster Bay	Point Clear	Weeks Bay N	West End Beach
Salinity	2 weeks	0.89	14.71	ND	15.15	20.99
Salinity	1 month	0.80	14.02	ND	14.53	20.33
Salinity	2 months	0.68	10.22	10.34	11.57	19.16
Salinity	3 months	0.46	7.92	8.21	8.68	17.30
DO	2 weeks	73.12	72.49	ND	71.43	91.56
DO	1 month	73.43	72.14	ND	74.21	88.23
DO	2 months	70.02	75.44	74.07	74.86	84.89
DO	3 months	70.04	77.52	56.86	76.85	69.55
Min DO	2 weeks	3.04	1.95	ND	1.24	1.54
Min DO	1 month	3.41	1.70	ND	1.88	2.29
Min Do	2 months	3.16	2.57	2.76	2.04	2.35
Min DO	3 months	3.15	2.83	1.19	2.52	1.70
Temp	2 weeks	18.30	29.71	ND	29.96	27.58
Temp	1 month	24.26	29.82	ND	29.99	27.86
Temp	2 months	26.29	29.67	28.34	29.69	27.93
Temp	3 months	27.33	28.68	27.19	29.02	26.09

Table B18. Spring water quality metrics gathered from weather stations for each collection site. Salinity (psu), Dissolved Oxygen (DO) (mg/L), and Temperature (Temp) (°C) are averaged over the timeframe listed. Minimum Dissolved Oxygen (Min DO) (mg/L) is the lowest 5th percentile over the timeframe listed.

Water Quality Metric	Timeframe	Car Ferry Marsh	Fort Morgan	Oyster Bay	Weeks Bay N	Weeks Bay S
Salinity	2 weeks	1.79	1.79	6.54	0.33	1.40
Salinity	1 month	1.72	1.72	8.05	0.99	2.02
Salinity	2 months	5.40	5.40	10.18	2.44	4.30
Salinity	3 months	7.63	7.63	11.78	4.58	6.80
DO	2 weeks	108.28	108.28	91.89	106.99	104.34
DO	1 month	104.68	104.68	91.65	102.68	104.28
DO	2 months	104.83	104.83	90.76	96.25	106.18
DO	3 months	103.79	103.79	91.82	97.70	107.31
Min DO	2 weeks	9.15	9.15	5.02	8.00	7.30
Min DO	1 month	9.38	9.38	3.75	8.00	7.60
Min Do	2 months	8.83	8.83	4.03	6.60	7.90
Min DO	3 months	8.48	8.48	4.26	6.20	7.90
Temp	2 weeks	16.82	16.82	14.48	22.14	23.28
Temp	1 month	15.22	15.22	14.76	19.37	19.66
Temp	2 months	14.53	14.53	14.77	17.82	17.62
Temp	3 months	14.62	14.62	14.51	17.54	17.01

Appendix C Residual Plots for Fig. 3.1

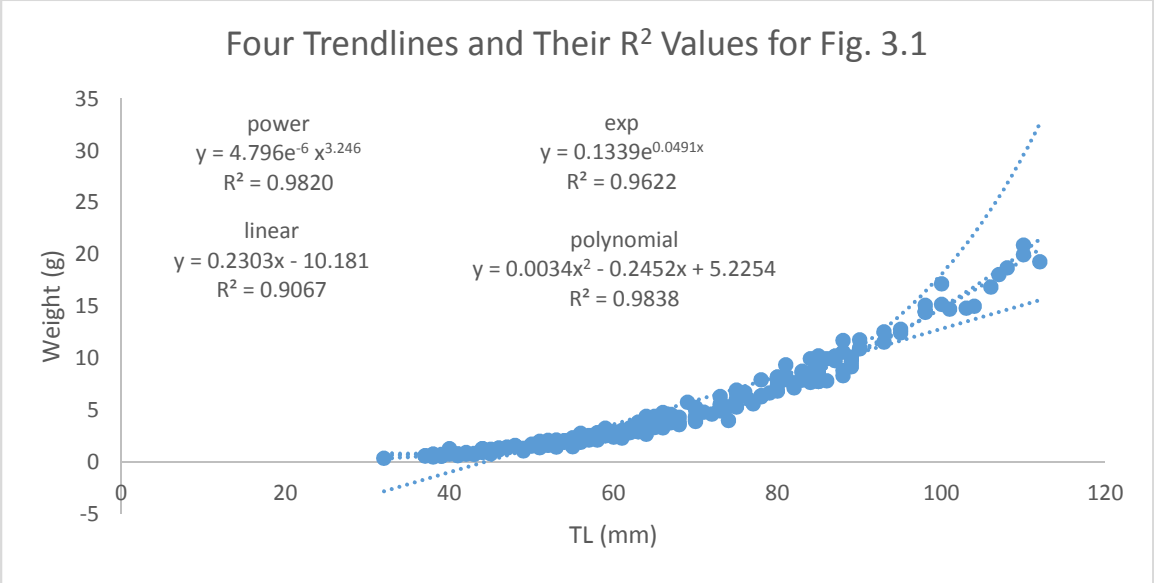

Figure C1. Four Trendlines and their R^2 Values for Fig. 3.1. Power trendline was the chosen best fit trendline.

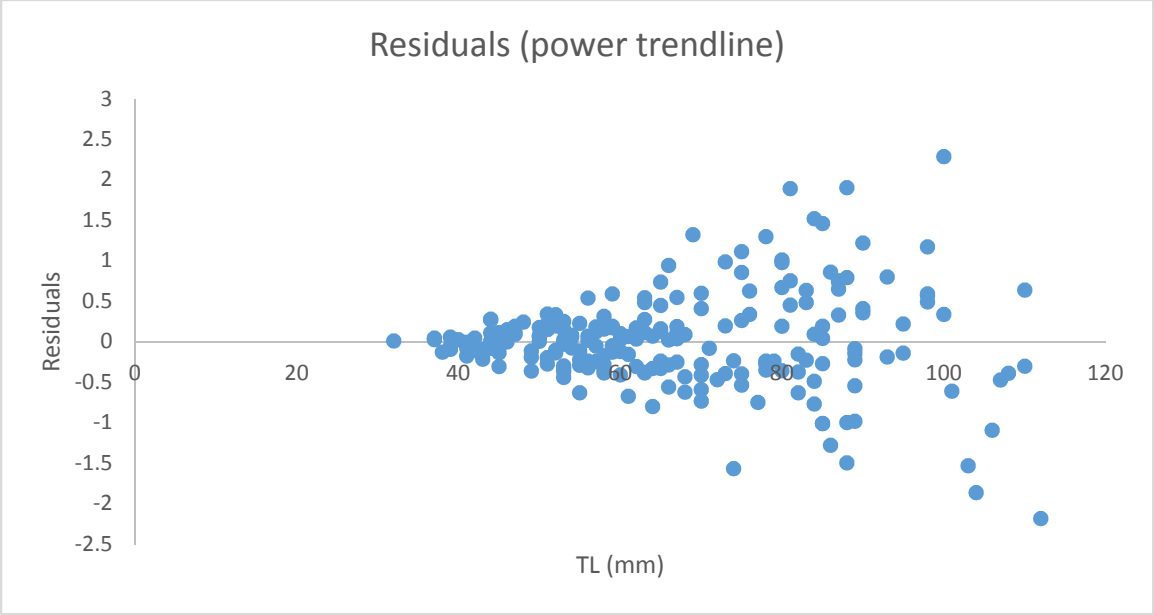

Figure C2. Residuals Plot for the Power Trendline. This was the chosen best fit trendline.

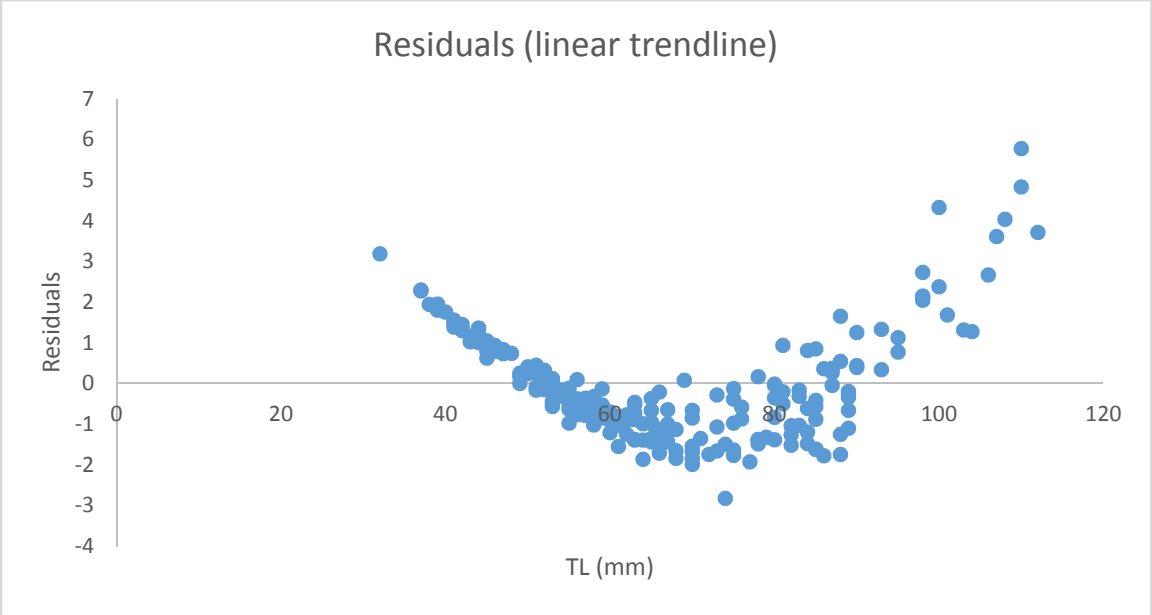

Figure C3. Residuals Plot for the Linear Trendline

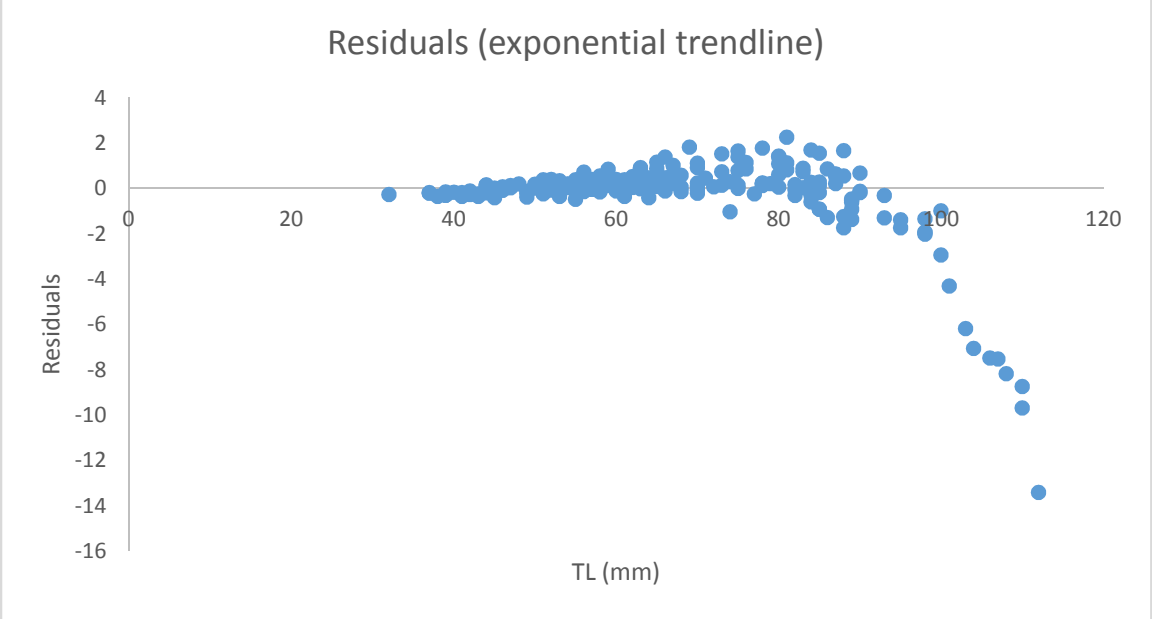

Figure C4. Residuals Plot for the Exponential Trendline

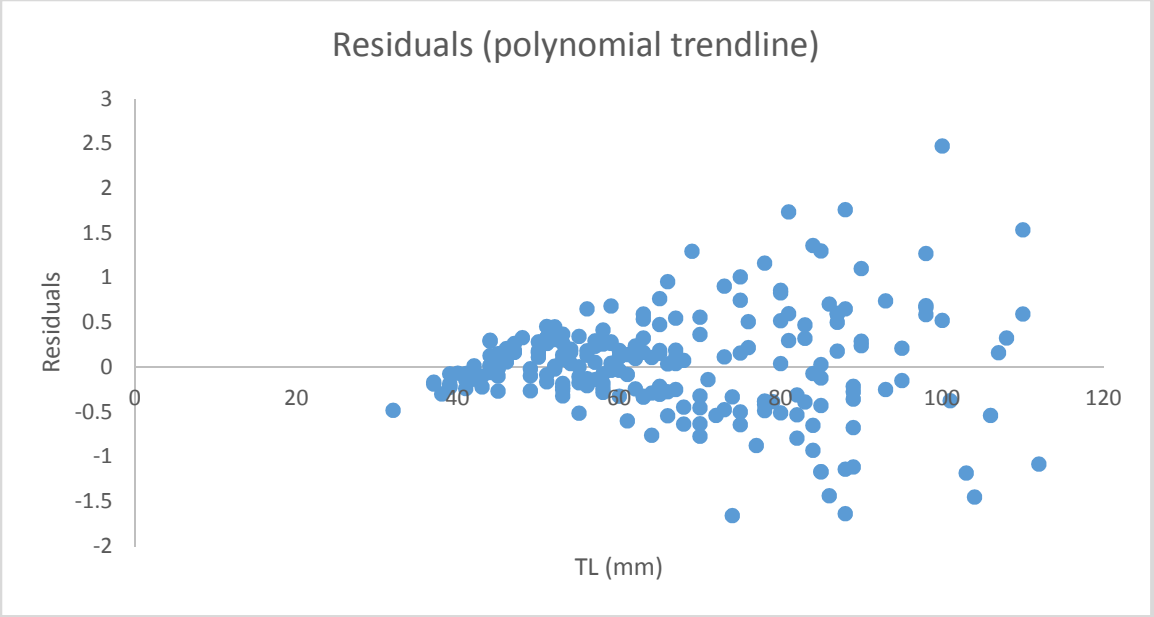

Figure C5. Residuals Plot for the Polynomial Trendline

Table C7. Four Trendlines and their R^2 and AIC values for Fig. 3.1

Model	Equation	R^2	AIC
Power	$y = 4.796e^{-6}\ x^{3.246}$	0.9820	-870
Exponential	$y = 0.1339e^{0.0491x}$	0.9622	-322
Polynomial	$y = 0.0034x^2 - 0.2452x + 5.2254$	0.9838	1181
Linear	$y = 0.2303x - 10.181$	0.9067	1408

Appendix D Relationship Plots for *Fundulus grandis* Condition Metrics

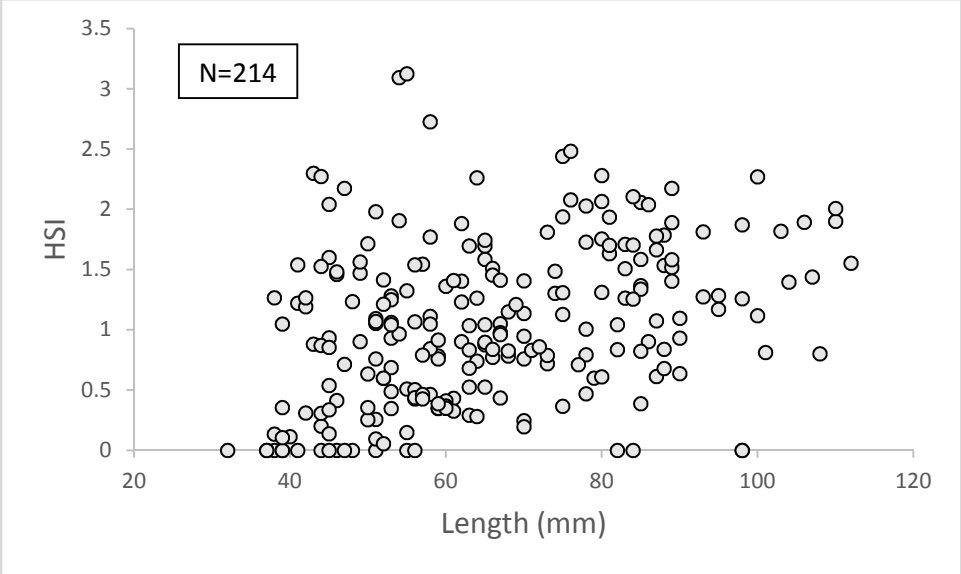

Figure D1. *Fundulus grandis* length vs hepatosomatic index. Sample size is listed in the upper left.

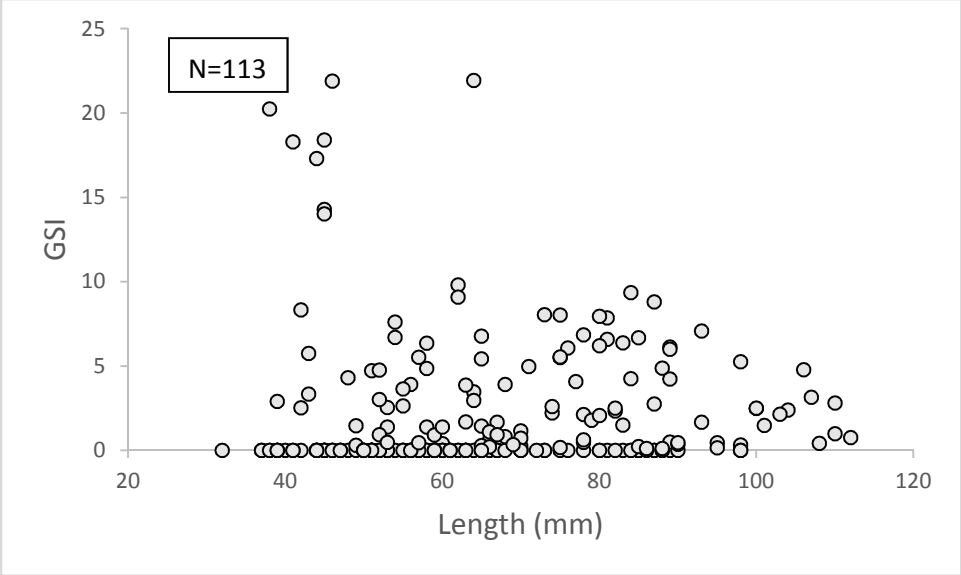

Figure D2. *Fundulus grandis* length vs gonadosomatic index. Sample size is listed in the upper left.

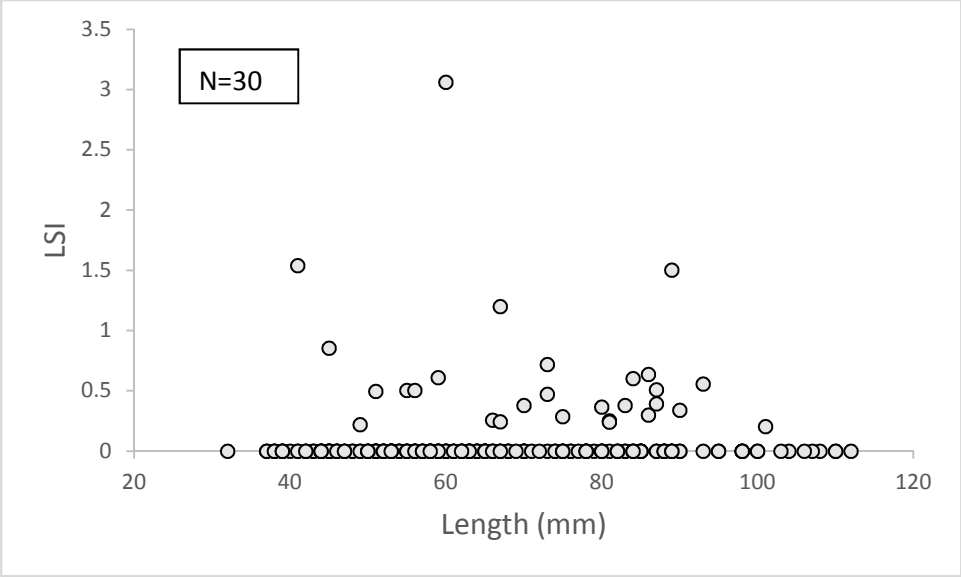

Figure D3. *Fundulus grandis* length vs lipo-somatic index. Sample size is listed in the upper left.

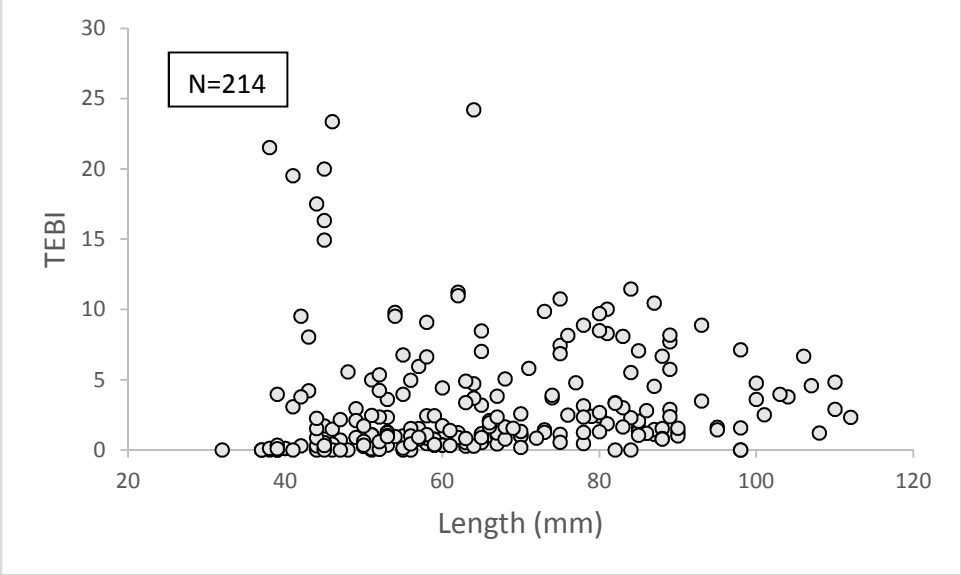

Figure D4. *Fundulus grandis* length vs total energy bodies index. Sample size is listed in the upper left.

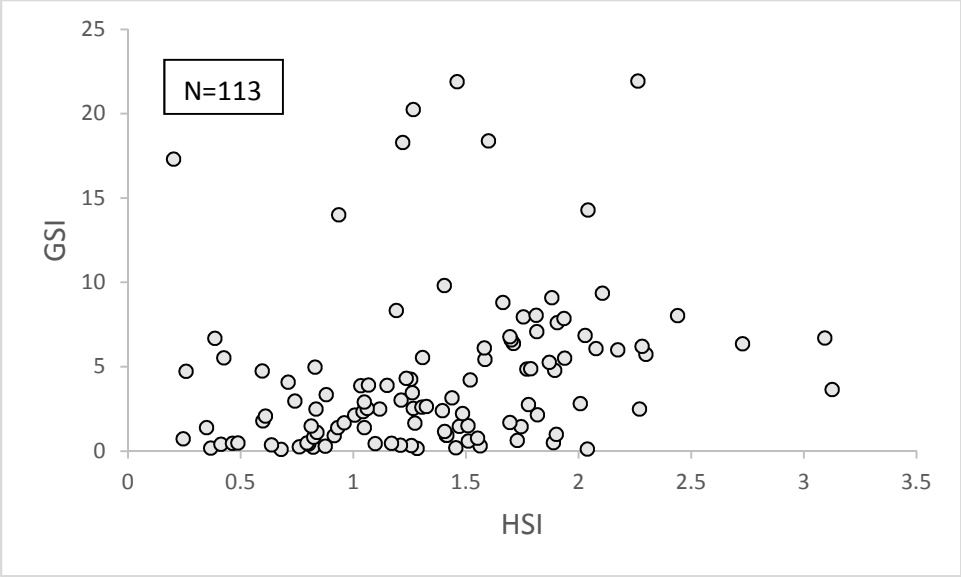

Figure D5. *Fundulus grandis* hepatosomatic index vs gonadosomatic index. Sample size is listed in the upper left.

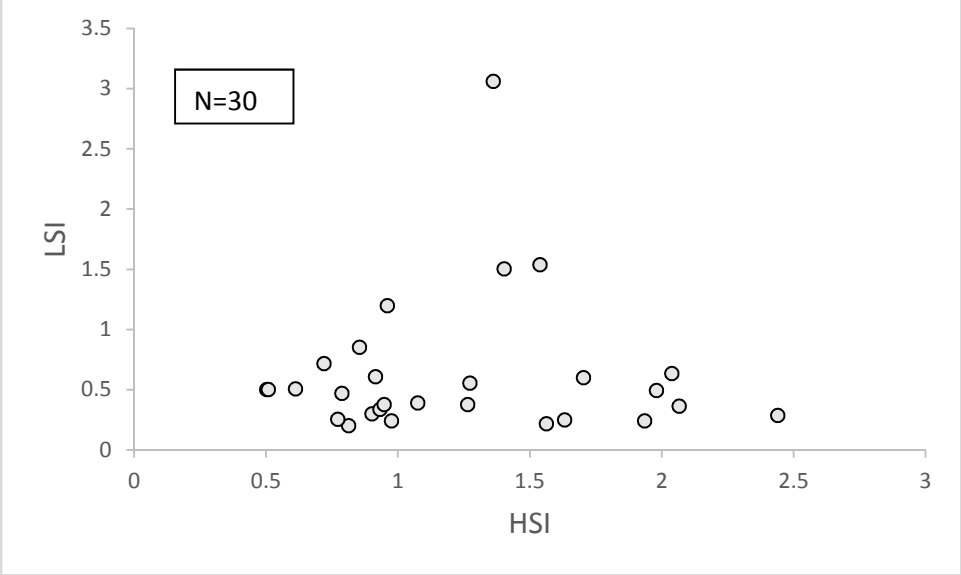

Figure D6. *Fundulus grandis* hepatosomatic index vs lipo-somatic index. Sample size of is listed in the upper left.

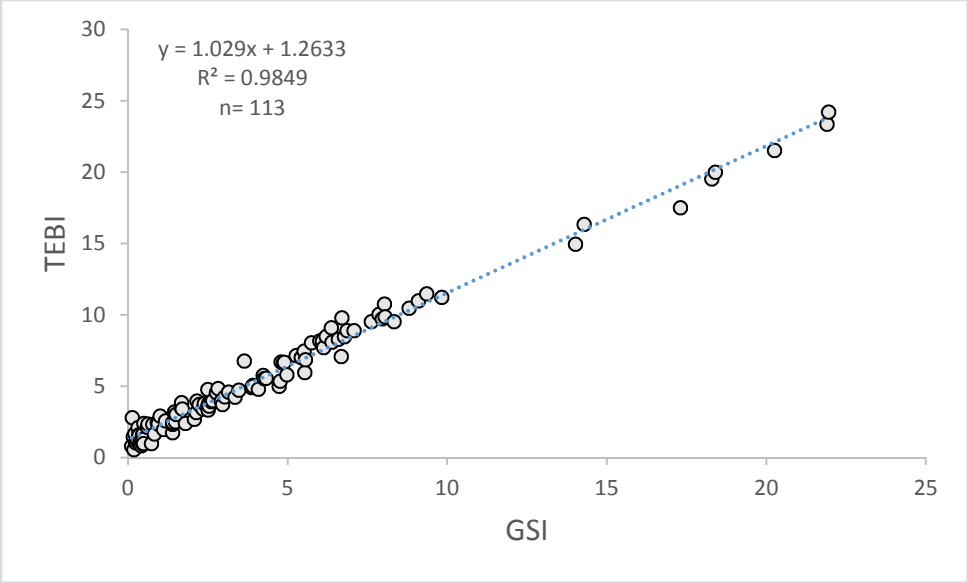

Figure D7. *Fundulus grandis* gonadosomatic index vs total energy bodies index. Sample size is listed in the upper left. A linear trend line, listed in upper left, was fitted to the data.

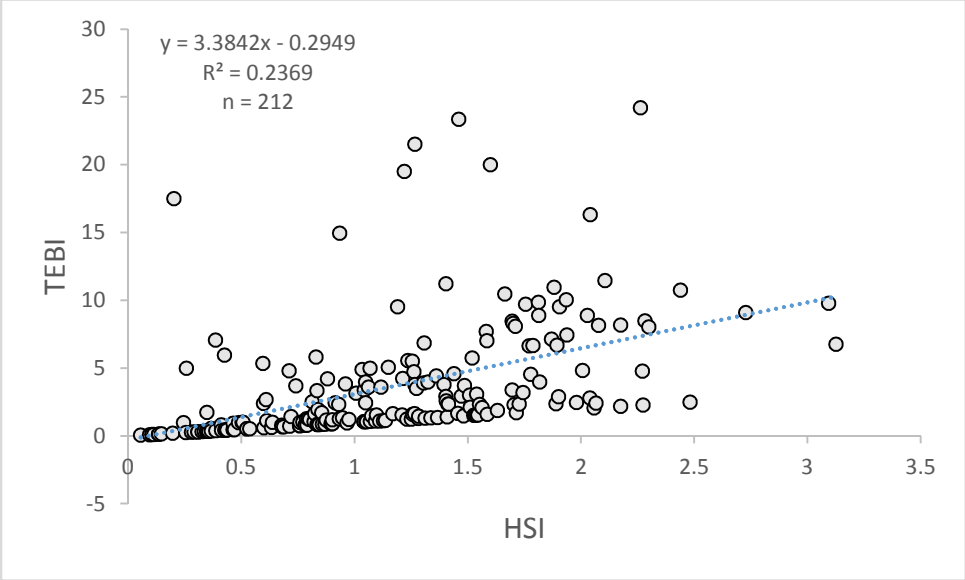

Figure D8. *Fundulus grandis* hepatosomatic index vs total energy bodies index. Sample size is listed in the upper left. A linear trend line, listed in upper left, was fitted to the data.

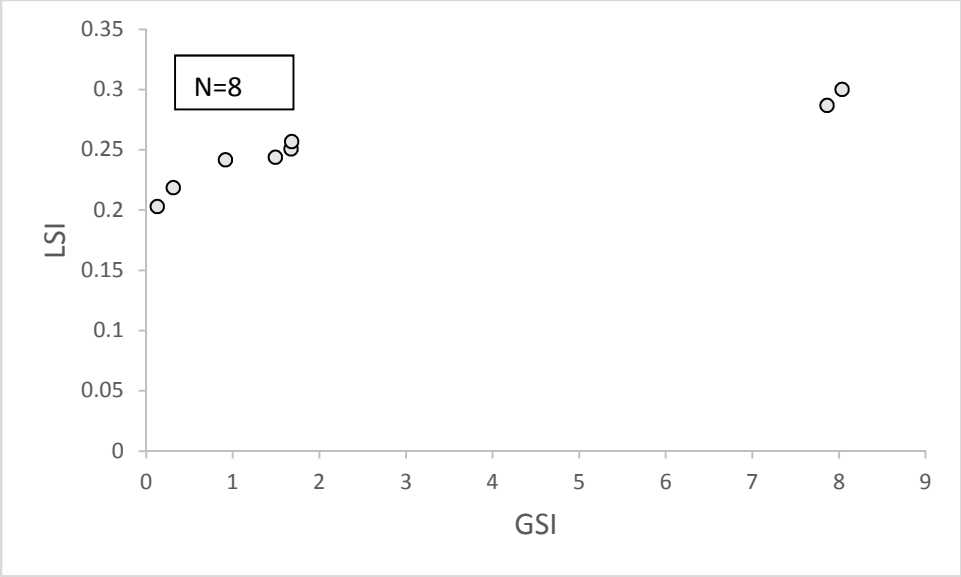

Figure D9. *Fundulus grandis* gonadosomatic index vs lipo-somatic index. Sample size is listed in the upper left.

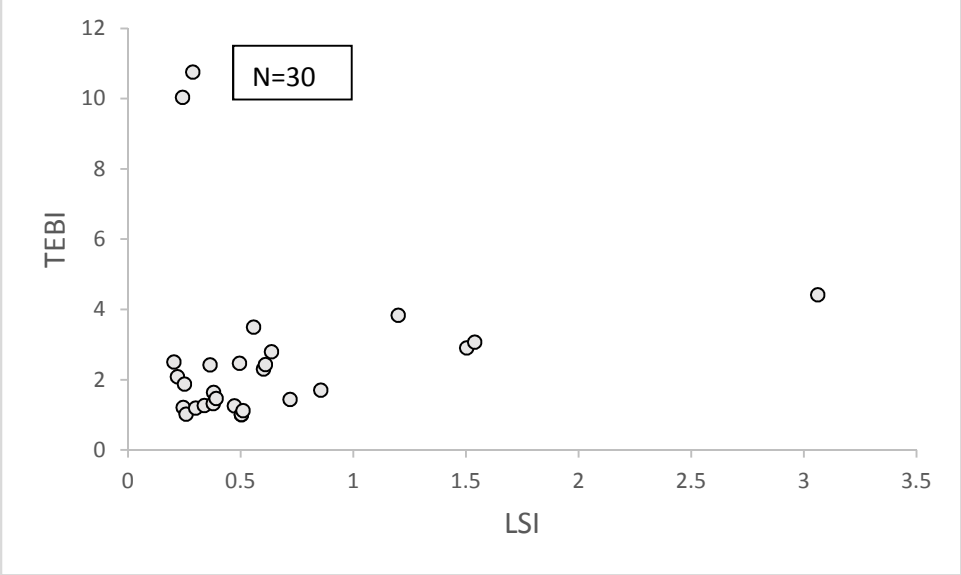

Figure D10. *Fundulus grandis* lipo-somatic index vs total energy bodies index. Sample size is listed in the upper left.

BIOGRAPHICAL SKETCH

BIOGRAPHICAL SKETCH

Name of Author: Nicholas A. LaBon

Place of Birth: Portland, Oregon

Date of Birth: August 2nd, 1991

Graduate and Undergraduate Schools Attended:
Augusta University, Augusta, Georgia
University of South Alabama, Mobile, Alabama

Degrees Awarded:
Bachelor of Science in Ecology, 2016, Augusta University
Master of Science in Ecotoxicology, 2021, University of South Alabama

Awards and Honors:
βββ Honor Society at Augusta University 2015-2016
Research Assistantship at University of South Alabama 2018-2020